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REFLEX ACTIVITY WITHIN THE SYMPATHETIC NERVOUS SYSTEM

HENRY G. SCHWARTZ[1]

From the Department of Anatomy, Harvard Medical School, Boston

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The present report is concerned with a series of experiments on reflex activity within the sympathetic nervous system. It has long been evident that many sympathetic phenomena occur in response to stimulation of sensory fibers which pass into the central nervous system, the impulse being transmitted thence to autonomic efferent fibers. Whether similar reflexes may be mediated solely through the isolated sympathetic system has, however, been open to question. In view of the contradictory evidence and opinions obtaining in regard to this problem, it is advisable to analyze briefly the more significant literature before describing the present series of observations.

The study of the peripheral endings of sympathetic afferent fibers has centered almost exclusively in the vascular system. As early as 1863 Kölliker spoke of the sensory innervation of blood vessels, but produced no direct anatomical proof. Dogiel (1898) stated that all blood vessels bore sensory myelinated fibers; their endings were demonstrated by Woollard (1926). Gläser (1914) suggested that sensory fibres might carry pain impulses in vascular diseases. Leriche (1921) applied this concept clinically, popularising periarterial sympathectomy.

There has been a sharp division of opinion concerning the trophic centers of these afferent fibers. Dogiel (1895, 1896) recognized primarily sympathetic sensory neurones with their cell bodies in the autonomic ganglia. Kuntz (1913) has been one of his few supporters. Kölliker originally postulated a cerebrospinal origin for the sensory fibers, considering them to be distributed peripherally in the region of the sympathetic. Huber (1897) agreed to this premise, as did Langley (1896); the latter observed that "most of the afferent fibers (i.e., from the spinal ganglia) join the sympa-

[1] Fellow in Medicine of the National Research Council.

thetic by white rami." In 1903 Langley admitted that possibly "a dozen or two out of many thousand (afferent fibers) can have their trophic centers in the autonomic ganglia."

Dogiel (1895, 1896) considered at least two distinct types of sympathetic neurones. Ramon y Cajal (1905, 1911) recognized three, and Michailow (1911) claimed the presence of nine types. Müller (1912) suggested that all sympathetic neurones were morphologically similar, but differed somewhat in the structure and disposition of their dendrites, according to the demands of the organs innervated by them. This distinction was overlooked by Carpenter and Conel (1914) whose anatomical preparations led them to conclude that all sympathetic ganglion cells were motor.

Ranson (1918, 1921) maintained that all visceral afferent fibers had a cerebrospinal origin. Rossi (1922) successfully impregnated, with the Golgi method, the Kölliker cells in the spinal ganglia, giving rise to fibers passing through the rami to the corresponding sympathetic ganglia.

It would seem, then, that Dogiel's opinion on the trophic center of sympathetic afferents has met severe contradiction. In all fairness it must be remembered that Dogiel himself was convinced of a cerebrospinal origin for the vast majority of afferent fibers; he felt only that some fibers came from discrete cells in the autonomic ganglia. Indeed, Hinsey (1928) admitted this possibility, and Wiedhopf (1925) claimed " . . . dass es . . . mit grösster Wahrscheinlichkeit auch sensiblen sympathischen Bahnen gibt."

Intrinsic efferent sympathetic fibers are well known. If, then, afferents, either intrinsic or extrinsic, exist, the occurrence of reflex activity may be expected. The existence of intrinsic sympathetic reflexes is still controversial.

Bernard (1864) working on the "submaxillary" ganglion, Sokownin (1874, 1877), on the bladder and the inferior mesenteric ganglion, and Francois-Franck (1894) on central stimulation of the ansa subclavia, reported sympathetic reflexes. Langley (1890, 1900) and Langley and Anderson (1894) claimed that the type of activity described was definitely not reflex, since no special afferent fibers were evident. Langley considered them to be "pseudo-reflex actions," similar to the axon reflexes obtained by Kühne (1886) in his classical experiments on the gracilis muscle of the frog. This concept was favored by Hryntschak and Spiegel (1928) who repeated some of the work of Langley and Anderson.

The phenomena of hyperalgesia and referred pain have been likewise explained as axon reflexes. Wernøe (1925) interpreted similarly the pigment reactions and peripheral vasoconstriction of fishes, following visceral stimulation. In attempting to clarify the problem of inflammation, Bruce (1910), Bardy (1915) and Krogh (1922) discussed the presence or absence of axon reflexes as being of paramount importance. Tower and Richter in

1932, working on electrical resistance of the skin, concluded "that the post-ganglionic neurones . . . are capable of some variety of activity independent of the central nervous system. . . . No true reflex arc . . . could be involved. . . . But an axon type of reflex taking place entirely in the periphery is possible."

In opposition to the concept of a pseudo-reflex, some evidence has been reported which would point to a tonic influence exerted solely by the sympathetic system. Popielski (1903) adopted this view for the coeliac ganglion; Nawrocki and Skabitschewsky (1891) for the inferior mesenteric ganglion; and Budge (1855), Braunstein (1894), and Langendorff (1900) for the superior cervical ganglion. Their evidence could not be confirmed, however, by Kovalewsky (1886), Schultz (1898) or Anderson.

More recently Boshamer (1925) believed he found a vasomotor tonic influence within the autonomic ganglia, and Schilf (1926) declared that "das vom Rückenmark abgetrennte Ganglion eine gewisse Selbständigkeit erlangt und den sonst vom Zentralnervensystem ausgeübten tonischen Einfluss auf die Gefässe bewirkt. Wenn man will, tritt das Ganglion als sekundärer Zentralapparat in Tätigkeit, während es für gewöhnlich nur eine untergeordnete Rolle spielt."

This statement received scant attention however, and Langley's influence is apparent in recent text-books of physiology, e.g., Starling, 1930, wherein it is concluded "that the sole use of these (sympathetic) ganglia is to serve as distributing centers."

From this brief survey of the literature the uncertain and controversial aspects of the nature of sympathetic reflexes are apparent. The present experiments were designed to re-investigate the question of the rôle of the sympathetic ganglia as autonomous reflex centers. Our experiments, which are presented in detail in the following pages, demonstrate that certain reflex impulses are mediated solely through the stellate ganglion requiring no central connections of the ganglion. Thus we have concluded that true sympathetic reflexes can be demonstrated,—results at variance with the prevailing concept of the nature of sympathetic reflexes as expressed by Langley and the majority of other investigators in this field.

METHOD. Cats were subjected to the following operations which were performed under ether anesthesia with aseptic precautions. In most cases the 4th, 5th, 6th, 7th, 8th cervical, and 1st and 2nd thoracic dorsal roots were sectioned extradurally on the right side. The centripetal connections of the right forelimb were thus abolished. Preservation of the anterior roots precluded the effects of muscular dystrophy. Some of the animals were subjected further to intra-thoracic sympathectomy on the right side, the chain being removed from above the stellate ganglion to the 6th thoracic. In one animal all the branches of the right stellate ganglion were sectioned except the grey ramus connecting it with the first thoracic nerve. Initial

unilateral resection of the dorsal root ganglia from the second lumbar to the first sacral was performed on two animals. Repeated observations were made at intervals from two to sixty days following operation. All lesions were verified at autopsy.

The skin-galvanic reflex (Tarchanoff, 1890; Richter, 1926, 1927, 1928, 1929) was selected as an indicator of reflex sympathetic activity.

The apparatus used to record changes of resistance in the skin is represented diagrammatically in figure 1. The current was led off from one or more dry cell batteries, and a sensitive microammeter was placed in the circuit.

Setting the known resistance at 28,400 ohms, the key, *K*, of the inner circuit was closed, and, by regulating the potentiometer, *P*, just enough current was allowed to pass through to give a maximal deflection of the ammeter, *A*. The cat was then brought into the circuit, and readings

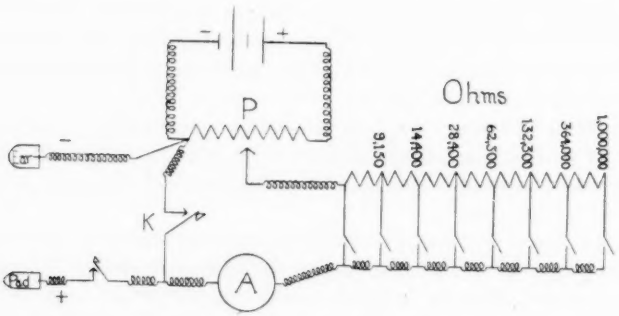

Fig. 1. Diagram of apparatus. For explanation, see text

could readily be obtained on the scale of the ammeter, to be subsequently converted into terms of resistance.

It was found that best contact, without mechanical interference, could be obtained by inserting the cat's paw into a rubber bag filled with kaolin-zinc sulphate paste; a zinc electrode was then placed in the bag, and the whole wrapped with elastic. By this means artefacts were excluded, which were found to be present at first when the electrodes were attached by adhesive. Another electrode was attached to an ear which had previously been pricked. Thus the main resistance in the circuit was at the pad.

With the animal in the circuit, no observations were made until a point of rest or minimum activity obtained, as indicated by the steady position of the indicator. Several types of stimuli were then applied: 1, deep pressure over the deafferented leg; 2, pinching the skin of areas with intact nerve supply; and 3, auditory stimuli (whistling, suddenly tapping the

table with a pencil). Visual perception of a hand or instrument moving in close proximity to the head was found to have an excitatory effect; accordingly care was taken to preclude optic stimuli.

RESULTS. I. *Changes of resistance in animals with dorsal roots sectioned.* Ten animals, with somatically-deafferented right forelimbs, were repeatedly examined. Only very deep pressure upon the deafferented leg gave a response; stimuli applied to the skin or superficial structures of the limb yielded no measurable effect. Figure 2 illustrates the results obtained. It may readily be seen that a definite decrease in the resistance occurred following application of any effective stimuli used, except when stimulation

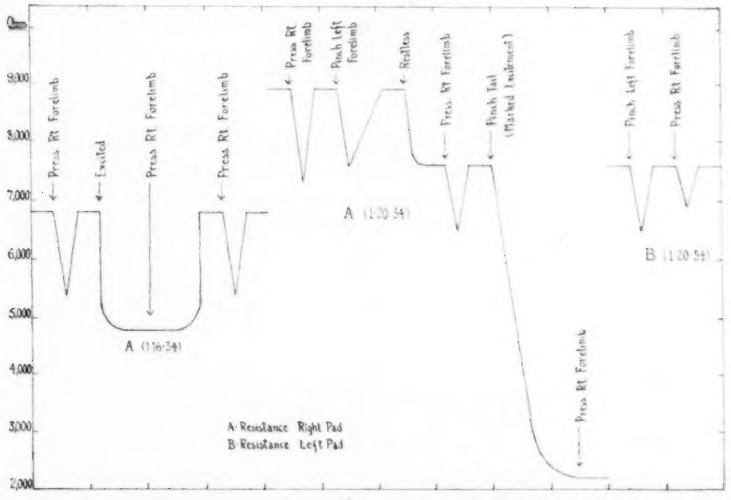

Fig. 2. Typical responses obtained in animals following section of dorsal roots (C_4-T_2) on the right side. In this and subsequent figures the ordinates are the measured resistance; the abscissae represent approximately the relative time relations for each response.

was applied at the height of excitation. It was not feasible to determine the time relations accurately. It may be stated, however, that any stimuli not arising from the deafferented limb gave rise to longer-enduring responses; on the other hand, deep pressure over the right forelimb produced not only an immediate response, but, upon release of pressure, an immediate return to a position of rest, as well.

In most cases strong auditory stimulation (loud whistle) as well as painful stimuli elsewhere applied (tail-pinch) gave a distinctly greater decrease of resistance than did deep pressure over the affected limb. Further, application of pressure over the deafferented leg at the height of marked

auditory or painful excitation resulted in no observable increment of reaction.

Resistance of the pad of the left (normal) forelimb was also measured. In contrast to the deafferented limb, superficial cutaneous stimulation alone gave an effect. It is noteworthy that deep pressure over the right (deafferented) limb resulted in a decrease of the resistance of the left fore-pad, with an immediate return to a resting state. The mechanism causing this response probably consists of afferent sympathetic impulses passing down the right thoracic chain to below the level of the second thoracic; there the impulse can pass through the intact dorsal roots and cross over and upwards to the opposite side.

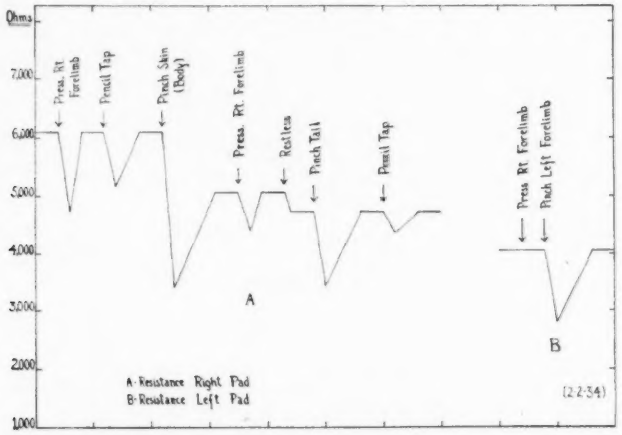

Fig. 3. Responses obtained in an animal with right fore-limb deafferented, and all connections of the right stellate ganglion sectioned, except one grey ramus to T_1.

II. *Observations after removal of all branches of the stellate ganglion except one grey ramus, after dorsal root section.* In one animal, previously deafferented and examined along with others of that group, the chain above and below the right stellate ganglion was resected, and all the rami were severed except the grey ramus to T1. The right forelimb, accordingly, received its sole sympathetic supply from the stellate ganglion through one grey ramus, the ganglion being isolated from all other nervous connections.

Electrical resistance was measured and the results were almost identical with those obtained prior to the second operation, that is, skin resistance of the right fore-pad decreased in response to the stimuli described above (fig. 3). Resistance of the left fore-pad, however, while decreasing in response to painful and psychic stimuli, did not shift when deep pressure was applied to the affected right limb. This was to be expected, since the central connections of the stellate had been removed.

III. *Observations following removal of the thoracic sympathetic and section of the dorsal roots.* Two animals were subjected to a second operation consisting of resection of the thoracic sympathetic chain, including the stellate ganglion on the right side. In these animals deep pressure over the right

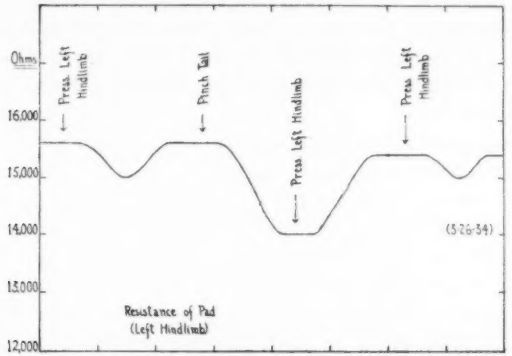

Fig. 4. Typical responses obtained in animals following dorsal root ganglionectomy (L_2–S_1) on the left side. The reaction time is increased.

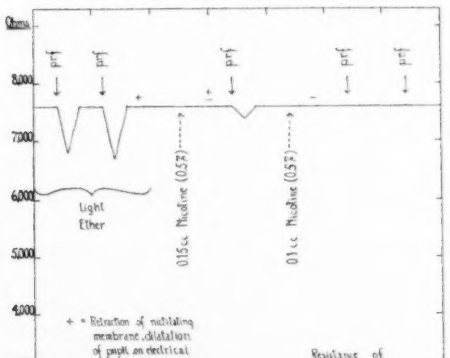

Fig. 5. Results in an animal with deafferented right forelimb, showing the effect of nicotinisation.

forelimb showed no change in the resistance of either the right or left pad. Psychic stimuli and pressure applied elsewhere had no effect on the resistance of the right pad, whereas resistance on the left showed the usual decrease. It should be noted that, following sympathectomy, the skin resistance, previously about 7,000 to 8,000 ohms, increased markedly to between 15,000 and 16,000 ohms.

IV. *Observations after section of the dorsal root ganglia.* Two animals were subjected initially to unilateral section of the dorsal root ganglia from L2 to S1. The hindlimb was chosen in these cases, for technical reasons: the ganglia in this region are much more readily removable. With peripherally deafferented hindlimbs prepared in this way, the resistance of the pad was measured. Here again a response could be elicited, similar in every way to that encountered in the forelimb, but differing in time relations (fig. 4). There was an appreciably longer latent period as well as a delayed return to the resting stage.

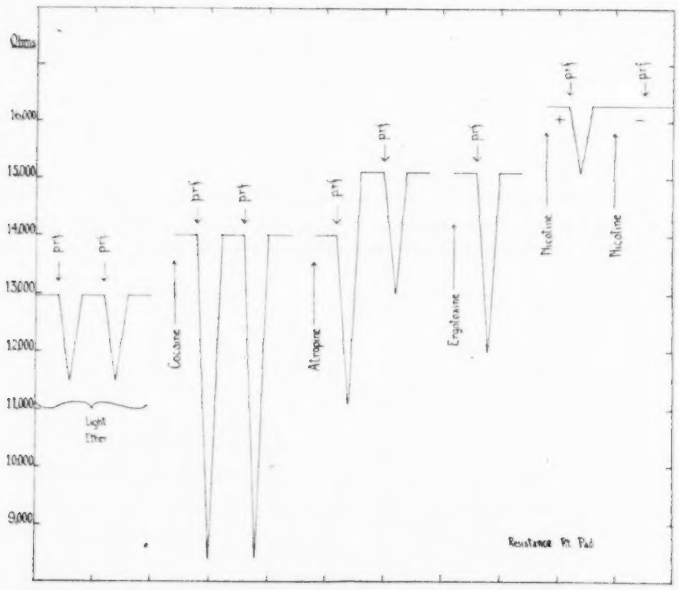

Fig. 6. The effects of drugs on the responses obtained in an animal with deafferented right forelimb. The plus and minus signs are used as in figure 5.

V. *Effects of drugs upon the response of the deafferented limb.* It was found, in two animals with the forelimbs deafferented, that the typical response could be elicited under light ether anesthesia. In one of these a solution of nicotine (0.2 cc. of 0.5 per cent solution) was injected intravenously. Figure 5 illustrates the result. Upon disappearance of the response of the nictitating membrane and pupil to stimulation of the cervical chain, there was concomitant abolition of the skin galvanic reflex.

Intravenous injection of cocaine definitely increased the response in one animal (fig. 6). There was some decrease thereafter upon injection of

atropine and ergotoxine. An insufficient dose of nicotine was next given, and a slight response was obtained. When, however, an amount of nicotine sufficient to abolish the reaction of the membrane and pupil was given, the galvanic reflex disappeared as well.

DISCUSSION. I. *The indicator.* Whatever may be the basis for the skin galvanic response, it is very probable that it is a response dependent upon sympathetic activity. This view is supported by the fact that the effect is increased following injection of cocaine (fig. 6) and disappears upon sympathetic denervation (p. 599).

The effector mechanism involved is apparently a complex one. From his own observations, Richter (1929b) concluded that "palmar resistance is dependent on the sweat glands." So far as the results herein reported are concerned, the slow reactions obtained in the hindlimb (cf. p. 600) may well be explained on this basis. It is problematical, however, whether the rapid reactions in the forelimb are exclusively dependent on sweat gland activity. The possibility that they might be due to muscular action potentials may be dismissed, since reversal of the electrodes produced no concomitant reversal of the microammeter indicator. The decrease in the galvanic response, and the increase in the basal resistance following injection of ergotoxine point towards the possibility of vasodilatation playing a rôle.

II. *Differences in the responses.* In all cases except those sympathectomized, pressure over the peripherally deafferented forelimb resulted in a rapid response; release of the stimulus was followed by a prompt return to the resting state. On the other hand, stimulation of normally innervated parts, while resulting in an immediate response, showed a more sluggish return. This applied to auditory as well as to painful stimuli. The delay in recovery may be explained as a prolonged after-discharge, or a temporal dispersion of the nerve impulses.

As may be seen in the figures, a slight response occurred if moderate excitation was present, none when the animal was extremely excited; the usual response, however, occurred when a position of rest and minimal sympathetic tone was restored. It is not surprising to find no detectable response to stimulation applied during intense excitement, the effector organs having probably reached their maximal activity.

III. *Reflex or pseudo-reflex?* The reactions persist after dorsal root section (p. 597) and disappear following sympathectomy (p. 599). The sympathetic alone is therefore sufficient for the responses. Their abolition upon injection of nicotine strongly suggests the presence of a synapse, thus making quite improbable the mechanism of an axon-reflex through the postganglionic fibers.

It might be contended that the reaction is a "pseudo-reflex" (see Langley, 1900b), the afferent nerve fiber involved shedding a branch to the postganglionic efferent cells lying in the stellate, and its main trunk passing

directly to a cell body in the dorsal root ganglion. This contention, however, is untenable, for the galvanic response persists after ablation of the dorsal root ganglia (fig. 4). It should be added that in all cases sufficient time was allowed to elapse to ensure complete degeneration. The foregoing evidence, then, is entirely in accord with the concept of an intrinsic sympathetic reflex, the synapse being located in the stellate ganglion.

The fact that deep pressure was necessary to elicit a response in the peripherally deafferented limbs leads to the assumption that the receptor apparatus lies in the deep tissues, probably in the arteries, but possibly in the muscle or in the periosteum. Hinsey (1928) described sensory fibers

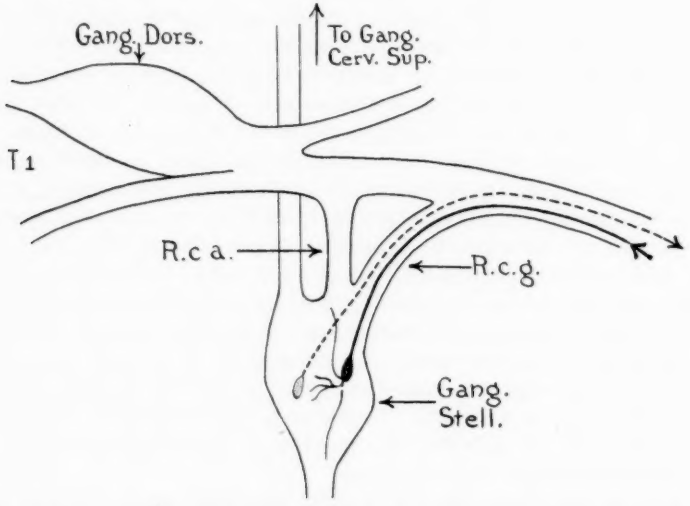

Fig. 7. Schema of mechanism responsible for the reflex, showing an afferent fiber with its cell body in the stellate ganglion (*Gang. stell.*). *R.c.g.*—ramus communicans griseus. *R.c.a.*—ramus communicans albus.

which he said "are probably associated with muscle pain and are those which are stimulated when a blood vessel is cut or handled."

From the present experiments it must be concluded that the cell bodies lie somewhere on the path between the receptor organ and the stellate ganglion. They might well be located in the ganglion itself (Dogiel, loc. cit.). What course may the afferent fiber pursue to reach its center of origin? Figure 7 represents the probable arrangement of the fibers concerned. It is obvious that at least some fibers should pass through the grey ramus (*R.c.g.*, fig. 7), since in the animal with all the other branches of the stellate cut, the reflex was still obtained (p. 600).

It is generally stated that most of the visceral afferent fibers pass through the white rami. It is not impossible to conceive that in addition there are intercalated sensory sympathetic neurones which pursue this path. Upon such an assumption there may be established a connection between the dorsal root ganglion cells and the neurones which are responsible for the transmission of afferent impulses from the periphery to the stellate ganglion. Indeed, the presence of an intercalated neurone may lend support to Ramon y Cajal's idea (1911) concerning the source of "pericellular arborisations" in the dorsal root ganglion.

The fact that responses have been shown to be submaximal when mediated solely through the sympathetic system, and that the reaction time was more rapid in the animals with intact dorsal root ganglia, supports the contention that by far the great majority of fibers have their cells of origin in the spinal ganglia. It is evident, however, that there are some sensory fibers which belong intrinsically to the autonomic nervous system.

SUMMARY AND CONCLUSIONS

Changes in skin resistance in the pad of a cat's forepaw occur in response to reflex activity of the sympathetic nervous system. In the present study it is shown that a certain fraction of these reflex impulses are mediated solely through the stellate ganglion; that is, they are true sympathetic reflexes and require no central connection of the stellate ganglion. The afferent fiber carries impulses probably from the blood vessels or deeply lying tissues of the forelimb, through the grey ramus, to its cell of origin in the stellate ganglion. There synaptic relations with the efferent neurones occur.

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A DIFFERENTIATION BETWEEN PHOTOSENSITIZED AND ULTRA-VIOLET EFFECTS ON FROGS

H. F. BLUM AND C. R. SPEALMAN[1]

From the Division of Physiology, University of California Medical School, Berkeley, California

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Certain similarities may be pointed out between the effects of ultra-violet radiation on living systems and the effects produced in the same systems by visible light when a photosensitizer is present (photodynamic action). It would be impractical here to review all the cases in which the same observable end result follows treatment by either method; a few exceptions will be discussed below. It has been frequently assumed that the two phenomena are similar or identical in nature, the photosensitizing dyes simply serving to extend the ultra-violet effects into the visible spectrum.

As a matter of fact, a very considerable difference appears to exist between the two phenomena; whereas the ultra-violet effects can occur either in the presence or absence of molecular oxygen, the photodynamic effects occur only in the presence of O_2. In tables 1 and 2 is given a summary of experiments which point out this difference. While numerous, such experiments have been thus far confined to simple animals or simple biological systems, and it has seemed advisable to continue such studies on higher animals to assure ourselves that the same general rule holds. It may be mentioned that, as pointed out by Blum (1932), the photosensitizers commonly used may bring about reactions in non-living systems which do not require O_2, though such effects have never been demonstrated in living systems. The question becomes particularly important when it is realized that the use of photosensitizers has frequently been carried into the field of therapeutics with the idea of replacing or enhancing the activity of ultra-violet light. One of us (Blum, 1933b) has pointed out that even though photosensitizers may find therapeutic use, it should not be assumed that they produce the same effects as ultra-violet radiation if such a fundamental difference exists.

EXPERIMENTAL. *The effect on frogs.* When first exposed to light, photosensitized frogs show general signs of excitation, jumping about

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TABLE 1

*Photosensitized effects in presence and absence of molecular oxygen**

(Wave lengths longer than 3500 Å)

SYSTEM	PHOTOSENSITIZER	EFFECT			INVESTIGATORS
		In presence of O_2	In absence of O_2	Conditions	
Bacteria "Bacterium vulgare"	Rose bengale Methylene blue Phenosafranine	Killed	None	?	Jodlbauer and Tappeiner (1905)
Erythrocytes (mammalian)	Eosine Erythrosine Rose bengale Methylene blue Na Dichloranthracene disulphonate	Hemolyzed	None	Vacuum	Hasselbalch (1909)
	Eosine	Hemolyzed	None	Vacuum, illuminating gas, H_2	Schmidt and Norman (1921)
	Eosine Erythrosine Rose bengale Hematoporphyrin	Hemolyzed	None	?	Eidinow (1930)
	Eosine	Hemolyzed	None	CO	Blum and McBride (1931)
Skeletal muscle (frog)	Rose bengale	Stimulation to contraction	None	H_2, N_2	Lippay (1930)
	Eosine	Stimulation to contraction	None	N_2, vacuum	Spealman and Blum (1933)

Bacteriophage	Methylene blue	Inactivated	None	Vacuum	Clifton (1931)
	Methylene blue	Inactivated	None	N_2, vacuum	Perdrau and Todd (1933)
Blood clot					
Fibrinogen, calcium and prothrombin mixture	Methylene blue	Clotting prevented	Normal clotting	Vacuum	Baumberger, Bigotti, and Bardwell (1929)
Enzymes					
Invertase	Erythrosine	Destroyed	None	H_2	Jodlbauer and Tappeiner (1905)
Diastase	Erythrosine	Destroyed	None	H_2	
Toxins					
Ricin	Erythrosine	Inactivated	None	H_2	Jodlbauer and Tappeiner (1905)
Protozoa					
Spirostomum	Eosine Quinine	Killed	None	Vacuum	Straub (1904)
Viruses					
Herpes Vaccina	Methylene blue	Inactivation	None	Vacuum	Perdrau and Todd (1933)

* Numerous other experiments give evidence less conclusive but indicating the necessity of O_2.

rapidly and often making movements of the legs to wipe the region of the eyes. These movements may continue for some time, often being intermittent with periods of quiescence; they disappear when the animal is removed from the light. After exposure for some time the animals may show prostration and paralysis followed by death some hours later even though the animal is removed from the light. These symptoms correspond with the description given by Hausmann (1923), the original reference to which we have been unable to find. It is difficult to describe the effects more definitely, since they vary in degree with the kind and concentration of the sensitizer, and with the intensity of the light. Thus, frogs sensitized by an injection of 10^{-7} mols rose bengale or eosine per gram of frog reacted instantly when exposed to direct sunlight, whereas frogs sensitized with

TABLE 2

Effects of ultra-violet radiation (wave lengths shorter than 3500 Å) in presence and absence of O_2

SYSTEM	EFFECT			INVESTIGATORS
	In presence of O_2	In absence of O_2	Conditions	
Bacteria	Killed	Killed	H_2	Bie* (1905)
Erythrocytes (mammalian)	Hemolyzed	Hemolyzed	Vacuum	Hasselbalch (1909)
Enzymes invertase	Inactivated	Inactivated	H_2, CO_2, N_2	Jodlbauer and Tappeiner (1906)
Skeletal muscle (frog)	Contracture	Contracture	N_2 vacuum	Spealman and Blum** (1933)

* Bie summarizes a number of previous investigations by other workers, some of which give conflicting results, and points out criticisms of the methods.

** In some of the experiments the ultra-violet effects appear to be somewhat reduced in the absence of O_2. In our experience with skeletal muscles and with intact frogs no difference in effect was observed.

the same quantity of fluorescein reacted only after 15 to 30 minutes and then much less violently; exposure of the rose bengale frogs to sunlight for a half hour resulted in death some hours later, whereas frogs injected with eosine and exposed to a 500-W Mazda lamp under the conditions described below were not killed by several hours' irradiation.

Laurens (1933, p. 480) makes the statement that "The sensitizing reactions are specific," and furthermore that "only eosine, chlorophyll, and certain derivatives of hemoglobin have so far been found effective *in vivo*, and the only marked sensitizer for higher animals is hematoporphyrin." Such statements need at least a certain amount of qualification. We have attempted to sensitize frogs with fluorescein, eosine, rose bengale, acridine yellow, acridine orange, neutral red, hematoporphyrin, and methylene

blue; the quantities injected were approximately 10^{-7} mols per gram. Of these only the first three, which all belong to the fluorescein series, and hematoporphyrin could be shown to produce definite sensitization to light. One reason for this was evident on inspection of the frogs after injection of the dye; the rose bengale and eosine frogs were a bright red, the fluorescein frog showed a yellow color on the belly surface, but viewed from above the presence of the dye could only be observed by the fluorescence of the eyes. The amount of photochemical reaction taking place must depend upon the quantity of light absorbed by the dye, and if the dye does not penetrate to the skin where it may be reached by light, it cannot be effective; this seems to be the case with those dyes which we found ineffective. Moreover, if the wave lengths which are absorbed by the dye are more or less the same as those absorbed by the skin pigments, we must expect that the amount of light reaching the dye in the skin will be greatly reduced. The fact that the fluorescein frog appears to have approximately the same color as a normal frog, although the dye seems to be distributed to the skin as shown by the yellow color given to the lighter belly skin, indicates a certain correspondence between the absorption spectrum of the dye and that of the frog's skin, whereas the rose bengale frog is distinctly red because of the lack of this correspondence. This may account in part at least for the greater activity of rose bengale as a sensitizer for frogs. However, there are undoubtedly other factors concerned. The hematoporphyrin frogs showed very little color but considerably more activity when exposed to light than the fluorescein frogs, though less than the eosine or rose bengale frogs.

No observations have as yet been accurately made in which the photosensitizing activity of the dyes has been compared with the amount of light absorbed by the dye under given experimental conditions. Even though exact correlation were not found in such experiments, it would not necessarily mean that the sensitizing reactions were "specific." On the other hand, an examination of table I should indicate very definitely the existence of a common type of reaction for the various sensitizers. Blum (1930 and 1934) has pointed out the possible importance of factors not connected with or only indirectly connected with the actual photochemical reactions, i.e., the toxicity of the non-irradiated dye and the toxicity of its breakdown products, which may modify the total picture of photodynamic action, and which may be considered in a sense as specific.

Excitation which cannot be readily distinguished from that described above, results from treatment of non-sensitized frogs with quartz-mercury arc radiations. We have never continued this treatment long enough to observe whether the frogs might be killed by it.

Site of action. Frogs with the brain and spinal cord destroyed did not show the excitation described above; animals with only the brain de-

stroyed showed some excitation, though relatively little. We may conclude, then, that the movements are produced reflexly due to stimulation of sensory nerves.

The effect of O_2 lack on photosensitized animals. In each of these experiments, one sensitized and one non-sensitized animal were simultaneously subjected to the same radiation in the same chamber. The apparatus employed is diagrammed in figure 1. The animals were enclosed in a chamber which could be evacuated, or through which air or nitrogen could be passed; a sheet of plate glass served as cover for the chamber through which the

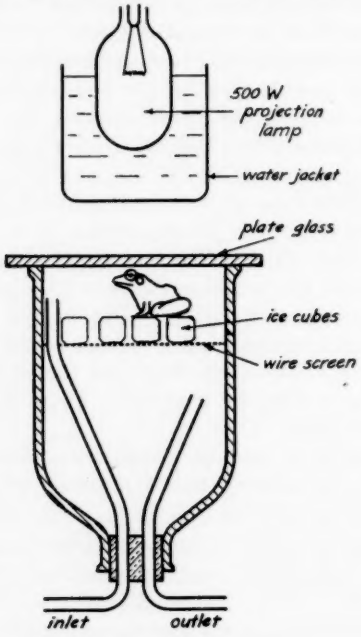

Fig. 1

light reached the animals. The radiation was obtained from a 500-W projection lamp, the filament at about 15 cm. from the frogs, which was surrounded by a water jacket so arranged that the light passed through a minimum of 3 or 4 cm. of water before reaching the chamber; a constant flow of water was maintained through this water jacket. With this arrangement the chamber reached a considerable temperature after long periods of irradiation, and since it was not desirable to increase the distance of the lamp from the animals by interposing a greater thickness of water screen, it was found convenient to keep down the temperature by placing

cubes of ice in the chamber and allowing the frogs to rest directly on these. Non-sensitized frogs remained unaffected under these conditions for as long as 2½ hours. Eosine, rose bengale, and hematoporphyrin were employed as photosensitizers.

When an atmosphere containing O_2 was maintained by the passage of a stream of air through the chamber, the sensitized frog always showed the above described symptoms of sensory stimulation within a few minutes; the control animal remained inactive.

To remove the O_2 from the frogs the chamber was evacuated to a pressure of about 25 cm. of mercury and then allowed to fill with commercial N_2 which had been passed several times through alkaline pyrogallol. The N_2 was allowed to pass through the chambers for some minutes at about 1 cm. Hg above atmospheric pressure and the evacuation then repeated. This procedure was repeated five times in the course of an hour, after which it was assumed that the frogs were saturated with N_2. No change in behavior of the animals could be observed during this treatment; respiratory movements continued and the animals occasionally moved about spontaneously. The flow of N_2 through the chamber was continued through the experiment. If the light was switched on, it was without effect on either the sensitized or non-sensitized frog, even though the irradiation was continued for over one hour. If air was then admitted and allowed to pass through the chamber for some minutes, the sensitized frog again reacted to the light in the usual manner.

The effect of O_2 lack on the ultra-violet effect. For the irradiation of non-sensitized frogs by ultra-violet a similar chamber was used but of smaller dimensions, allowing space for only one animal, the plate glass being replaced by a sheet of fused quartz. The light source was a 220 V. Cooper Hewitt type quartz mercury arc operated on a line voltage of 110 V. The arc was placed at about 30 cm. from the chamber; cooling was obtained by interposing a quartz walled filter 2 cm. in thickness through which a stream of distilled water was passed, and by ice in the chamber. With this arrangement, stimulation of the animals occurred within a few seconds after exposure to the light; exactly the same results were obtained in air and in N_2 applied in the same manner as described above.

A criticism of the experiments on sensitized animals might have been offered, namely, that the ability to respond to sensory stimuli is abolished by the reduction of O_2 tension to such a low point as was done in our experiments. This criticism is, however, met by the fact that under exactly the same conditions of virtual absence of O_2, the frogs do respond to sensory stimulation produced by ultra-violet light.

Discussion. It would be obviously impossible to extend experiments of the type herein described to all examples of photodynamic action. Only in selected cases can the experiment be made as it is not always possible

to deprive the organism of oxygen without producing effects which obscure the photodynamic picture. Since all experiments which appear to have been critically performed lead to the same conclusion (see table 1) it may be assumed to be universally true. While the number of experiments on ultra-violet effects are less numerous (see table 2) they are nevertheless quite conclusive.

Relatively few experiments appear to have been carried out with the purpose of comparing the effects of ultra-violet and photodynamic action. We may cite those of Levy (1929) and Videbech (1931) for destructive lesions of mammalian skin; Campbell and Hill (1924) for effects on frog's mesentery, Lippay (1929, 1930, 1931) and Spealman and Blum (1933) for the stimulation of skeletal muscle, Howell (1921) on the coagulation of fibrinogen, and Gassul (1920) for effects on internal organs following irradiation of the intact animal. Levy, Campbell and Hill, and Gassul find strikingly similar results; whereas Howell, Videbech, Lippay, and Spealman and Blum find very distinct differences. It seems probable that the similarities in effect when they occur are actually determined by a common site of action rather than by the similarity of the photochemical reactions. Thus in the intact frog, the photochemical reaction must be confined to the surface of the body and whatever the products of the reaction, the only immediate effects we might expect to see would be symptoms of sensory stimulation such as described above.

Schantz (1921) and Clark (1922) have suggested mechanisms for the explanation of photosensitization in which it is assumed that the ultra-violet action is due to a photoelectric effect, the photosensitizer shifting the threshold of the photoelectric effect into the visible region. Whatever the mechanism of ultra-violet action, the fact that a second molecule, i.e., O_2, is necessary for photodynamic effects indicates that we are dealing with a photochemical reaction and not a photoelectric effect. Blum (1933a) has pointed out a thermodynamically possible photochemical relationship between the ultra-violet and photodynamic effects based upon the production of H_2O_2 as the active agent in both cases. This hypothesis has not, however, found support in the experiments of Spealman and Blum (1933). Thus it appears that we must, at least for the present, assume a definite dissimilarity between ultra-violet and photosensitized effects.

SUMMARY

Photosensitized frogs irradiated with visible light exhibit similar reactions to those produced in non-sensitized frogs irradiated with ultra-violet light.

The two effects may be differentiated, however, by the fact that ultra-violet effects occur in the presence or absence of O_2, but the photosensitized effects only in the present of O_2.

Thus the two processes must be considered as essentially different.

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PASSAGE OF FLUID AND CERTAIN DISSOLVED SUBSTANCES THROUGH THE INTESTINAL MUCOSA AS INFLUENCED BY CHANGES IN HYDROSTATIC PRESSURE

E. S. NASSET AND A. A. PARRY

From the Department of Vital Economics and Department of Physiology of the Medical School, University of Rochester

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The point of departure for the experiments herein described is a paper published by Wells (1931) on the "absorbing force" of the intestine. He found that at a certain pressure below atmospheric the apparent absorption of fluid from an isolated loop of intestine ceased. The pressure applied was less than atmospheric, expressed in terms of centimeters of the solution with which the gut was filled, and varied in different animals between -8 and -26 cm. From these experiments it was concluded that the absorption of water from isotonic solutions of sodium chloride and dilute solutions of glucose ceased whenever the intraintestinal pressure was brought to a certain negative value within the above range. This pressure, characteristic for each individual being studied, was expressed as ". . . an exact measure of the absorbing force of the intestine." In a later paper, after making determinations of the colloid osmotic pressure of the lacteal lymph, Wells (1932b) reached the conclusion that "The findings indicate that the absorbing force of the intestine is due to the osmotic pressure, exerted against the semipermeable epithelial membrane of the intestine, of that fraction of the total serum proteins to which, on the average, the walls of the blood capillaries of the villus are permeable."

In this laboratory some of the problems concerned with the secretion of the small intestine (Pierce, Nasset and Murlin, 1931–1932), (Parry and Nasset, 1933), (Pierce and Nasset, 1934), (Nasset and Pierce, 1934) have received some attention and it occurred to us that a repetition of some of Wells' experiments with a little different viewpoint might lead to information of some interest for secretion as well as absorption. It seemed reasonable that the process of secretion of fluid and dissolved substances into the lumen of the intestine might readily influence the results obtained in the type of experiment just described. The mechanical irritation to the mucosa afforded by the wire coil, which is inserted into the loop to prevent its collapse at negative pressures, might easily stimulate the secretory process even though the animal were anesthetized. It had been found

that the insertion of a catheter into the transplanted intestinal loops of our operated dogs results in an increased secretion. This is especially noticeable during the first hour of experiment.

It was thought necessary, therefore, to approach this problem with the idea that there are at least two distinct processes involved, namely, absorption and secretion.

METHODS. The methods employed were essentially those described by Wells (1931) with some few modifications. Any one interested in the details of the method should consult Wells' description of it. It consists essentially in an acute intestinal loop preparation so arranged that at constant pressure, usually below atmospheric, changes in volume of the system can be determined continuously in a burette attached to the open end.

An aluminum helical coil was used to prevent collapse of the intestinal loop. The glass cannulae at the ends were made fast by winding adhesive tape over both wire and glass. One cannula was formed of a T-tube so that a thermocouple could be inserted into the lumen for the determination of the temperature of the contents of the loop. This modification was adopted after it was discovered that the temperature of the loop being used exerted a very marked influence upon the results obtained.

The most satisfactory method of keeping the loop warm and moist, without raising appreciably the temperature of the whole animal, was to enclose it in a chamber which fitted over the animal's abdomen. The opening in the bottom was large enough to pass down over the platform supporting the loop. On either side at the bottom were troughs through which water at any desired temperature could be circulated. In this manner it was possible to control the temperature of the loop.

In a number of experiments a thermo-stromhur was attached to a mesenteric vein. This instrument was similar to those described by Rein (1928). The heating was done with 60 cycle A.C. at 10 to 12 volts. In these experiments it was necessary to select a loop of intestine which was drained by a single large vein so that any fluctuations noted in blood flow could be said to have occurred in the loop which was under observation, Figure 1 gives some idea of the construction of the apparatus used in these experiments.

Rather large dogs ranging in weight up to 34 kgm. were used. They all received a vermifuge and were fasted the 36 hours immediately preceding the experiment. The anesthetic in all cases but two was sodium amytal given intraperitoneally. In the two instances in which ether was used, no remarkable differences in the results were obtained and hence the nonvolatile anesthetic was adopted because of its greater convenience.

The experimental procedure was as follows: After establishment of complete anesthesia the dog was placed belly up on an animal board, a

tracheal cannula inserted and the abdomen opened. A suitable section of small intestine was selected, opened at appropriate points, thoroughly washed out with the solution to be used and the aluminum coil carefully inserted and secured at either end by heavy ligatures. The supporting platform was placed as near to the body wall as possible to avoid stretching of the mesentery. After inserting the thermocouple the rubber connections were attached, to the burette at one end and to the waste overflow at the other, and the entire system freed of air by filling with solution from the burette. If the stromuhr was to be used it was put in place at this time. The moist chamber was closed and the meniscus in the burette

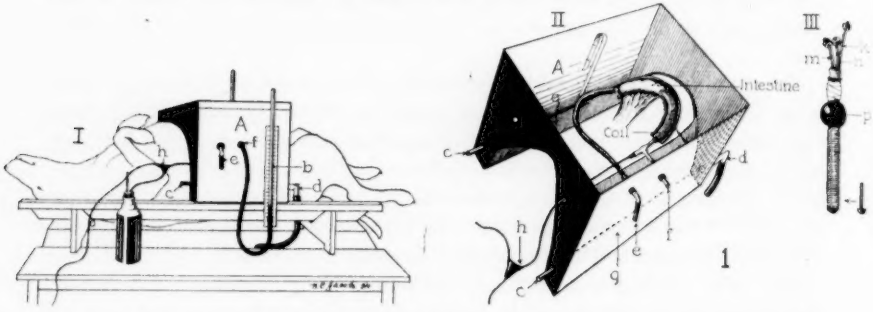


Fig. 1. I. *A*—moist chamber; *b*—burette and scale; *c*—warm water inlet; *d*—warm water overflow; *e*—waste overflow from intestinal loop; *f*—burette connection to loop cannula; *h*—loop thermocouple.

II. *A*—interior view of moist chamber. Part of the loop is cut away to show the helical coil used within the loop. The semi-circular platform supports the loop just above the belly wall. *g*—troughs on either side of the body which carry warm water for the regulation of temperature and humidity of the chamber.

III. Coil used on operated dogs with intestinal transplant. *k*—inlet for solution; *m*—outlet for solution; *n*—air tube for inflation of balloon; *p*—balloon.

Our thanks are due to Mr. N. C. Jacobs for the drawing of this figure.

set at any desired level below the platform supporting the loop. The changes in volume were recorded, and the meniscus brought back to its original position every 10 or 5 minutes or oftener, depending upon the rate of change. Besides the loop temperature, the temperatures of the air in the moist chamber and of the rectum were recorded at frequent intervals.

In the case of the operated dogs with chronic intestinal transplants the apparatus was slightly different but the principle of the experiment was the same as described above. Since the loops in these dogs were closed at one end the coil had to be constructed accordingly. A length of small bore brass tubing (1.5 mm. inside diameter) was inserted into the center of the coil and made fast to it at the end which was to be placed at the blind

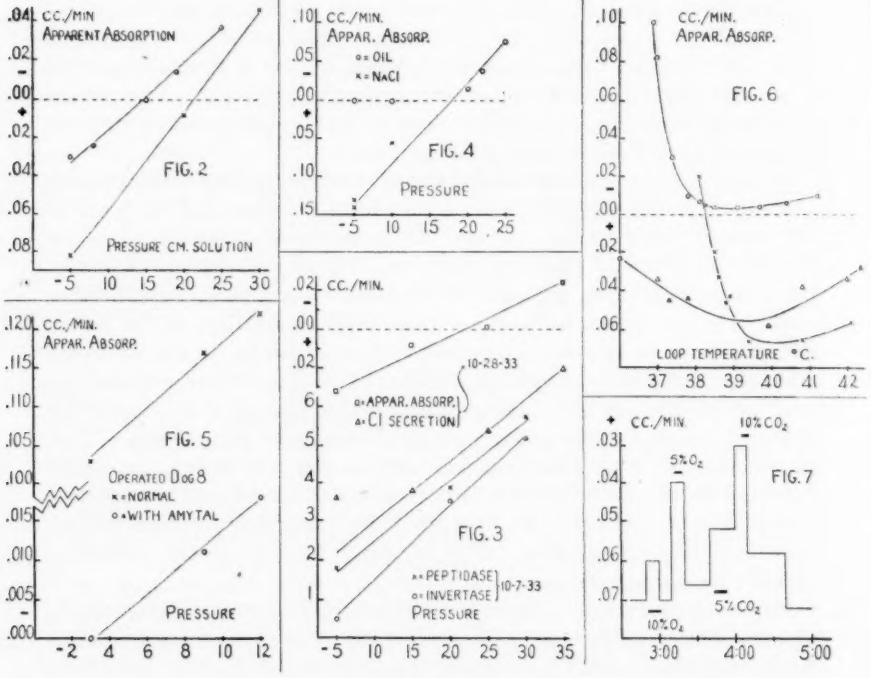
end of the loop. A hole in the tubing served to carry the solution to the far end of the loop. Another tube opened at the near end and served to drain the loop. It was possible also by this means to circulate fluid through the loop. The loop was maintained air-tight by means of a small rubber balloon made from the little finger of a surgical glove which was fitted over the center tubes. This balloon was made to fit just inside the opening of the fistula below the surface of the surrounding skin and was inflated sufficiently to prevent air from leaking into the system at the negative pressures employed.

RESULTS. *Apparent absorption and pressure.* The term "apparent absorption" is preferred for the changes in volume noted in the intestinal loop. The apparent absorption is expressed as plus or minus depending on whether the volume decreased or increased respectively. At a certain negative pressure the volume of the system will remain constant and this is called the point of zero apparent absorption.

Wells found that a linear relation existed between pressure and apparent absorption in the region between atmospheric pressure and the point of zero apparent absorption. Below this point there appeared to be no regular behavior with respect to pressure. Figure 2 shows two of four of our experiments which indicate that the linear relation exists in this region also. *A priori* there is no reason, if there is such a relation, why it should disappear at zero apparent absorption. It is apparent, as will be shown later, that this point at which the contents of the loop remain constant is a point of dynamic equilibrium at which the rate of passage of substances out of the gut is equal to the rate of passage of substances into the gut. That is, the rates of absorption and secretion in units of volume are equal. Above or below this point there is inequality and the apparent movement of fluid is in or out. In order to support this thesis it would be necessary to demonstrate that at least one of the processes was directly related to pressure changes in the gut.

Secretion and pressure. Because of our interest in secretion our attention was first turned to that phase of the problem. As shown by the work of Herrin and Meek (1933) the chloride concentration of the intestinal secretion is independent of volume within wide limits, and quite constant at about 0.7 per cent over relatively long periods of collection. The same has been found true, in this laboratory for hourly periods. By using a fluid in the intestinal loop which contained no chloride, e.g., glucose solution, it should be possible by appropriate sampling and analysis to gain some information regarding the rate of secretion at various pressures. There are certain enzymes also which are characteristic of this secretion and, although they are not secreted in constant concentration, a determination of the enzyme activity of the loop contents should give indicative results. Four experiments of this nature were carried out. Figure 3

illustrates the type of results obtained. Invertase activity was determined by taking an aliquot of the contents of the loop, incubating it at 38°C. in 1 per cent sucrose and determining the amount of reducing sugar present at the end of 48 hours. This necessitated the use of 0.9 per cent sodium chloride in the loop instead of the glucose solution used in the determination of the secretion of chloride. Erepsin was estimated in a similar manner except that the substrate was 5 per cent peptone and the amount of hydrolysis determined by formol titration. In the same figure is shown



Figs. 2 to 7

the amount of chloride recovered in another experiment from the loop contents at different pressures, along with the apparent absorption curve for the same experiment. Their parallel behavior is suggestive of a relation between intraintestinal pressure and secretion. It might be argued that secretion was constant and that the differences in chloride concentration reflected only the change in the ability of the intestine to reabsorb the salt at different pressures. If this were true for chloride it reasonably might be expected to hold true for other dissolved substances. The experi-

mental facts observed in the enzyme studies make the possibility of constant secretion and variable absorption seem very remote. The enzymes of the succus entericus are closely associated with the insoluble mucoid material that precipitates out of solution apparently as soon as it is formed. If a sample of fresh intestinal juice be centrifuged as soon as possible after collection, the supernatant fluid will be found to be practically devoid of enzymic activity. It is quite probable, therefore, that the greater enzymic activity of the intestinal contents at lower pressures is indicative of a greater production of the secretion which contains them. It seems reasonable to ascribe the changes in chloride concentration to the same cause. The experiments described in the following paragraphs give support to this thesis.

Absorption and pressure. In order to gain some information concerning the absorption of a dissolved substance at different pressures the experimental procedure was modified somewhat. Glucose solution of an accurately known concentration (about 4 per cent) was perfused through the lumen of the gut by means of an alternating siphon arrangement. The flasks at either end of the siphon were immersed in a small water bath for temperature control. The whole assembly could be set at any desired level below the loop of gut. After thoroughly washing the loop with distilled water the siphon tubes (small volume) were left filled with distilled water and 100 cc. of the glucose solution measured into one of the flasks. On removing the clamps from the tubes the sugar solution was made to flow through the gut into the other flask. This process was reversed when most of the solution had flowed through. The rate of perfusion was about 15 to 20 cc. per minute. This procedure prevented any great change in the concentration of sugar during the experimental period (20–30 min.). Table 1 contains the results obtained. The first experiments were done on an operated dog with an intestinal transplant. In the morning the procedure was carried out with the dog lying quietly on a table without anesthesia. In the afternoon the animal was given sodium amytal and the experiment repeated after complete anesthesia had been established. It will be seen at once that there is no indication of a decrease in the rate of absorption of glucose with decreasing intraintestinal pressures. It is evident that the anesthetic reduced the rate of sugar absorption to some extent, roughly about 16 per cent. The secretion of chloride was reduced proportionately much more. The failure of the initial chloride values to fall into line with the pressure curves is attributed to the initial burst of secretion alluded to above. The next was a sacrifice experiment which gave results in close agreement with those obtained with the operated dog. The rate of glucose absorption and chloride secretion is greater because the loop used was approximately three times the length of the one just described. In no case was there a trace of blood in the fluid recovered from the intestinal loops.

These experiments show that such a readily absorbable substance as glucose is absorbed at a fairly constant rate within the range of intraintestinal pressures employed in these experiments. It is felt that the reabsorption of chloride from the secretion of the intestine probably occurs also at a uniform rate regardless of small changes in pressure. Although it is impossible to distinguish between the water which arrives in the intestine by way of its secretion and that which is placed therein as salt or glucose solution, the evidence thus far supports the proposition that the volume changes observed at various negative pressures are the result of changes in the rate of secretion. Wells' interpretation of the significance of the

TABLE 1

Effect of hydrostatic pressure on glucose absorption

DATE	PRESSURE	GLUCOSE ABSORBED PER HR.	NaCl SECRETED PER HR.	LOOP TEMP.	REMARKS
1934	*cm.*	*mgm.*	*mgm.*		
5/4 a.m.	−5	296	44		No anesthesia. Dog IX. Jejunal loop
	−15	330	34	37.5*	transplant under right mammary
	−25		44	37.5	glands. Operated 11-29-33
p.m.	−5	229	16	38.0*	Amytal anesthesia. Dog IX. Same
	−15	288	14	38.0	loop as above
	−25	270	12	37.8	
5/9	−5	675	36	37.8†	Amytal anesthesia. Another dog. Acute
	−15	624	43	38.1	sacrifice experiment
	−25	663	62	38.0	
	−15	536	28	36.7	Effect of temperature on absorption of
	−15	560	41	40.3	glucose

* Temperature of an adjacent loop transplanted on opposite side of midline.

† Temperature of the loop used by means of thermocouple in the lumen.

point of zero apparent absorption, i.e., that absorption is abolished, is believed to be in error.

By filling the loop and system with a non-absorbable fluid it should be possible to gain further evidence as to the correctness of our interpretation of these results. An experiment was performed in the following manner: The platform supporting the loop was tilted at an angle of about 30° from the horizontal. At the lower end of the loop a Y-tube was inserted which had an enlargement blown at the intersection of the three arms. This enlargement served as a sump to collect the secretion at a certain stage of the experiment. To the two free ends of the Y-tube burettes were attached, one to contain 0.9 per cent sodium chloride solution and the other

to contain paraffine oil. The experiment was done in the usual manner first with salt solution, next with the entire system filled with oil and finally again with salt solution. Figure 4 shows the results. At pressures above the point of zero apparent absorption, with oil in the loop, the volume of the system remained constant, indicating a reabsorption of the secretion as fast as it was formed. Below this point the secretion was formed faster than it could be reabsorbed with the result that the volume of the system increased. The secretion was found in the sump at the lower end of the loop. The rates of negative apparent absorption determined with oil and those of positive apparent absorption determined with salt solution show a fairly good linear relationship to pressure.

Absorption and colloid osmotic pressure. If the absorption of fluid from the intestine is dependent upon the colloid osmotic pressure of the lymph, then the introduction into the lumen of a colloid with the same osmotic pressure should completely arrest the process. It is well known that a sample of an animal's own blood serum decreases in volume when introduced into a loop of intestine (Voit and Bauer, 1869) (Heidenhain, 1894). So far no one has described an enzyme in the small intestine which is capable of splitting native proteins with the possible exception of fibrin (Boldyreff, 1927). Therefore the serum proteins would continue to exert their osmotic effect and, indeed, Heidenhain states that the protein concentration of the serum in the loop is increased. Rabinovitch (1927) compared the rate of absorption of water and salt from 0.9 per cent sodium chloride solution with that from 6 per cent acacia in 0.9 per cent sodium chloride solution and found them identical. We performed three experiments in which we compared the rates of apparent absorption using solutions such as used by Rabinovitch and were able to confirm his results. Gum acacia, like starch, is easily hydrolyzed by dilute acid to yield reducing substances which in all probability are diffusible. Since the intestine of dogs secretes a very active amylase the possibility remained that in the acacia experiments this substance was rapidly hydrolyzed by some unknown enzyme and subsequently absorbed. Some 6 per cent solution of acacia was incubated at 38°C. for 48 hours with a relatively large volume of pure intestinal juice without the production of a trace of reducing substance. From this it is concluded that the acacia was probably unaffected and that, with the reduction of volume following absorption of water from such a solution, the colloid osmotic pressure of the contents of the intestine actually may have been increased.

The colloid osmotic pressure of lacteal lymph is approximately half that of the serum (Wells, 1932a). Therefore, if the osmotic theory of absorption as proposed by Wells (1931, 1932a, 1932b) be correct, blood serum or 6 per cent acacia, which is roughly equivalent in colloid osmotic pressure to blood serum, diluted with an equal volume of water should

suffice on the average to stop the absorption of water from such solutions when placed in the intestine.

According to Goldschmidt and Dayton (1919), and Rabinovitch (1927), sodium chloride solutions of concentrations as high as 1.2 to 1.5 per cent when placed in a loop of intestine are absorbed directly without any previous dilution. The total osmotic pressure exerted by mammalian serum is calculated from freezing point data to be about 6.7 atmospheres, which in turn is equivalent to a 0.95 per cent sodium chloride solution. In order to absorb, unchanged, sodium chloride solutions of 1.2 to 1.5 per cent the intestine must somehow overcome a difference in total osmotic pressure of from 1.7 to 3.9 atmospheres. It does not seem strange then that producing a change in intraintestinal pressure of the order of 20 to 30 cm. of water should have no measurable effect on absorption. The intent of this paper is not to throw the colloids out of court in connection with absorption from the intestine. Nevertheless, the facts indicate that the colloids are not the exclusive agency by means of which water is made to traverse the enteric epithelium.

Effect of anesthesia. Another type of experiment which discredits the osmotic theory was carried out with the aid of the operated dogs having intestinal transplants. The apparatus used was the same as that described in the study of glucose absorption with the exception that volume changes were observed instead of changes in chemical composition of the contents. The experiments were performed first with the dogs lying quietly on a table. They were then given sodium amytal intraperitoneally and the experiment repeated under complete anesthesia. This type of experiment was done once on each of three different operated dogs. Figure 5 illustrates the type of results obtained. It will be observed that the two curves are quite similar and parallel but that the one obtained in the normal state is considerably displaced in the direction of negative apparent absorption. On the basis of the osmotic theory, absorption could occur in this loop under these conditions only with the application of a pressure considerably in excess of atmospheric. On the other hand, the curve representing the same sort of experiment on the same loop under anesthesia crosses into the region of apparent absorption at a pressure below atmospheric. If the colloid osmotic pressure of the lymph were solely responsible for the absorption of water, then there appears to be no escape from the conclusion that the anesthetic caused the disappearance or inactivation in some manner of a large proportion of the lymph proteins. It seems much more plausible that the effect of amytal was chiefly a partial inhibition of the secretory activity of the loop. It is known that amytal and similar hypnotics inhibit certain secretions (Stavraky, 1931) (LaBarre and Wauters, 1932).

Effect of temperature. The temperature of the intestinal loop appears

to play an important rôle in its activity in this type of experiment.[1] Figure 6 shows the type of curve obtained by plotting the experimental data. Six of these experiments were done. This phenomenon has not been investigated to any great extent and therefore it cannot be stated with certainty whether absorption or secretion or both are affected by changes in temperature. The experiment dated 5–9–34 in table 1 gives the results of a glucose absorption study at three quite different loop temperatures with the intraintestinal pressure kept constant at −15 cm. of solution. There is an indication that the rate of glucose absorption is decreased at temperatures above or below body temperature. Although the components of the temperature effect cannot yet be identified its early recognition led to the institution of adequate methods for the control and measurement of loop temperature.

Pressure and blood flow. It was considered possible that the pressures employed to keep the intestinal wall snugly against the rigid wire coil in the lumen might be sufficient to interfere appreciably with the flow of blood to the gut and hence to affect its respiratory metabolism. In several experiments a thermo-stromuhr was attached to the vessel which carried all of the venous return from the loop under observation. Except for an initial diminution, which occurred in nearly all experiments, the blood flow did not show any great variations in relation either to the temperature or to the pressure which obtained in the loop.

In this connection the effect of breathing various gas mixtures was investigated in four experiments. With low oxygen tensions and high carbon dioxide tensions there was an inhibition of the apparent absorption (fig. 7) and hence an indication that asphyxia could play a rôle, but since the rate of blood flow was unaffected by the pressures used it is likely that the gaseous exchange of the gut was not greatly impaired.

Suitability of the method for study of secretion. The technic of observing the exchange of fluid and dissolved substances in the intestine at negative pressures lends itself quite well to the study of changes in the rate of secretion. The intravenous administration of pilocarpine will cause a change in the apparent absorption which is most easily accounted for on the basis of the secretagogue action of the drug. Epinephrine also will cause a rapid increase of short duration in the volume of the loop. This cannot be attributed to contraction of the loop because this drug is known to relax the muscles of the intestine. The effect of epinephrine may possibly be a result of the increased filtration pressure consequent upon the increased blood pressure. To our knowledge no other method is available for the study of changes in the rate of intestinal secretion in acute sacrifice experiments. This method has been used in the study of a number of drugs

[1] The first observation of this effect was made by one of us (A. A. P) and C. L. Ringe in the course of a student problem in the fall of 1932.

and extracts with partial confirmation by experiments on operated dogs (Nasset and Pierce, 1934).

Discussion. It is believed that the experimental facts presented in this paper demonstrate that, under the conditions imposed, the processes of absorption and secretion occur simultaneously at all times. If the rates of the two processes are not too widely different and at least one of them is dependent upon pressure, then it should be possible, within physiological limits of pressure, to find the point at which the rates are equal. This point usually is found at a pressure of 15 to 20 cm. of salt solution below atmospheric. Wells (1931) regards the process of absorption as stationary at this point and that this pressure is ". . . an exact measure of the absorbing force of the intestine." The data presented show that, as far as dissolved substances are concerned, the secretory process is dependent and the absorbing process is independent of pressure within the range investigated. It is manifestly impossible to distinguish what portion of the fluid in the intestine arrived there as secretion and, therefore, direct proof cannot be adduced that there is a constant two-way movement of fluid. On the other hand, the conception of other similar equilibria and the physiology of other glands of the body point to this interpretation as a probable one.

The fact that the intestine can readily absorb water from blood serum, 6 per cent acacia and 1.5 per cent sodium chloride solutions, indicates that the absorbing force of the intestine cannot be ascribed solely to the colloid osmotic pressure of the lacteal lymph. From the work of Rabinovitch (1927) it is calculated that the absorbing force of the intestine may be a matter of one or more atmospheres.

Finally it should be emphasized that in all studies of absorption from the gut the process of secretion must be taken into account and its possible influence upon the results given consideration.

SUMMARY

Experimental conditions are described under which it is shown that:

1. The absorption of glucose in aqueous solution is independent of intraintestinal pressure between the limits of 5 and 25 cm. of solution below atmospheric.

2. The secretion of sodium chloride and characteristic enzymes is increased in a linear fashion with decreasing intraintestinal pressure.

3. Increasing the colloid osmotic pressure in the lumen to that of blood serum does not show any influence upon the absorption of water and hence the colloid osmotic pressure of the lacteal lymph is inadequate to account for such absorption.

4. In operated dogs with intestinal transplants the apparent absorption is increased under anesthesia. This is best explained by an inhibition of secretion by the anesthetic.

5. The temperature of the loop under observation influences in a complex fashion the processes of fluid exchange occurring within it.

6. The intraintestinal pressures employed had little if any effect upon the blood flow through the loop.

7. The method described is adaptable to the study of changes in the rate of intestinal secretion in acute sacrifice experiments.

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RESPIRATORY ADAPTATION TO ANOXEMIA[1]

ALBERTO HURTADO, NOLAN KALTREIDER AND WILLIAM S. McCANN

From the Department of Medicine, University of Rochester School of Medicine and Dentistry and Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals, Rochester, New York

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Of the various mechanisms concerned with the adaptation to a low oxygen tension in the inspired air, those related to the respiratory system have been recognized as the most important, and of the more frequent occurrence. An increase in the pulmonary ventilation with a consequent rise in the alveolar oxygen tension, and a fall in the carbon dioxide tension have been observed by most investigators and interpreted as being of basic importance in the mechanism of adaptation. Much less attention has been paid to the rôle of structural changes in the lungs and alterations in the pulmonary capacity which may play a part in the response to anoxemia. The investigation of these latter factors, and their possible significance has been the object of the present study. Observations on the minute volume of breathing, the composition of the alveolar and expired air, the degree of anoxemia by direct examination of the arterial blood and the total pulmonary capacity and its subdivisions have been carried out in three healthy subjects at a simulated altitude of 16,400 feet in a low pressure chamber. These observations have been correlated to anatomical studies made of the lungs of guinea pigs subjected to the same experimental conditions.[2]

METHODS. Three healthy adult male subjects[3] were exposed to a barometric pressure of 419 mm. Hg, equivalent to an altitude of 16,400 feet,

[1] The expenses of this investigation were defrayed from a fund contributed by the Corning Glass Company, The Eastman Kodak Company, The American Grinding Wheel Manufacturing Association, The American Laundry Machinery Company, the Gleason Works, the Symington Co., and The Pfaudler Company.

[2] We wish to express our appreciation to the Secretary of the Navy for permission to carry out these experiments, and to Capt. E. W. Brown, M.C., U. S. N., Commander E. L. Gayhart, C. C., U. S. N., and to Lieutenant Hanson and Dr. James Hawkins of the Experimental Diving Unit, Navy Yard, Washington, D. C., for valuable coöperation and assistance.

[3] The age and physical characteristics of these subjects were as follows: Case 1. (N. L. K.) age 29 years, body height 187.7 cm., body weight 74.4 kgm. and body surface area 1.99 M^2. Case 2 (A. H.) age 32 years, body height 170.0 cm., body weight 72.4 kgm. and body surface area 1.83 M^2. Case 3 (W. S. Mc.) age 45 years, body height 171.5 cm., body weight 72.7 kgm. and body surface area 1.85 M^2.

in a low pressure chamber. This level was reached in an average of fifteen minutes in the three cases, and maintained unchanged for about two hours. The air in the chamber was replenished constantly so that there was a complete change every seven minutes which prevented accumulation of carbon dioxide and a reduction in the oxygen percentage. In all three experiments this was verified by analysis. Only in one instance, during the observation in case 1, the concentration of carbon dioxide in the chamber rose to a maximum figure of 0.31 per cent.

After a preliminary rest of about half an hour samples of alveolar air were obtained by the method described in a previous publication (1). The expired air was then collected for three minutes in a Douglas bag, and its volume accurately measured through a gas meter. The number of respirations during this time was recorded. Samples of the alveolar and expired air were stored in gas sampling tubes for subsequent analysis. The next procedure was to obtain arterial blood by direct puncture of the radial artery. This blood was collected without exposure to air and kept on ice in mercury sealed containers to be analyzed later. The red cell count was made using B. S. standard pipettes and the viscosity of the blood was measured in the Hess viscosimeter. The carbon dioxide content and the oxygen saturation of the arterial blood were determined in the manometric apparatus of Van Slyke-McNeill (2), and the amount of hemoglobin calculated from the oxygen combining power. Precautions were taken in all instances to adjust the pressure in the tubes containing the air and blood samples to the surrounding barometric pressure.

After a few minutes' interval the total pulmonary capacity and its subdivisions were determined in the recumbent position, using the methods and technique described in a previous communication (3). The measurement of the residual air by the oxygen dilution method of Christie, that is, by rebreathing a high percentage of oxygen during a few minutes, may be criticized on the grounds that the possible alterations in the residual volume produced by the low oxygen tension in the inspired air may be reversed during the rebreathing period (six minutes in all determinations), and thus an error would be introduced into the accurate measurement of this capacity. However, this appeared not to be the case and there are several factors to support this statement. A graphic tracing of the respirations during the rebreathing time did not show any alteration which would indicate a change in the resting respiratory level; the vital capacity and the reserve air before and after this period had the same values, and finally the oxygen consumption at the low pressure corresponded to that observed at sea level. Further indication of the accuracy of the determination was found in the close agreement of the results in repeated observations (duplicate determinations were made in all cases).

All the observations just mentioned had been previously made in the

chamber at the barometric pressure of sea level. Care was taken to use similar procedures (body position, length of observation, etc.) both at normal and reduced pressures. In cases 1 and 3 the observations at sea level were made immediately before those made at low pressure; in case 2 the preliminary investigations were made the day before.

The symptoms experienced by the subjects, and their clinical appearance, during the period of exposure to the low oxygen tension were briefly recorded. All were conscious of breathing and had a sense of mental dullness and fatigue. Evidence of cyanosis and dilatation of the capillaries of the skin and mucosa were present in all cases; more marked in subject 1, who also exhibited periodic respirations. There was an acceleration in the pulse rate almost immediately after the simulated altitude was reached,

TABLE 1

*Observations on the expired and alveolar air at sea level and at a barometric pressure of 419 mm. Hg (16,400 feet)**

	CASE 1 (N. L. K.)		CASE 2 (A. H.)		CASE 3 (W. S. MC.)	
	Sea level	Altitude	Sea level	Altitude	Sea level	Altitude
Expired air:						
Volume per minute (liters)	8.06	7.02	8.06	9.10	8.62	9.12
Respirations per minute	17	12	16	17	14	17
Tidal volume (liters)	0.47	0.59	0.51	0.54	0.60	0.54
CO_2 per cent	3.15	5.16	3.02	5.41	2.98	4.58
O_2 per cent	16.63	14.67	17.15	14.89	17.25	15.31
Alveolar air:						
CO_2 per cent	5.66	8.24	5.67	8.58	5.43	7.83
O_2 per cent	13.86	11.11	13.58	10.32	14.46	11.75
CO_2 tension (mm. Hg)	38.5	30.6	40.5	31.9	38.7	29.1
O_2 tension (mm. Hg)	94.2	41.3	97.2	38.4	102.9	43.7

* Observations made 30–40 minutes after being subjected to this pressure.

changing from 56 to 92 (case 1), from 76 to 90 (case 2) and from 80 to 88 (case 3) per minute. In no case was there marked respiratory distress, and the clinical appearance did not resemble that seen in moderate or severe cases of mountain sickness.

Four guinea pigs were exposed to the pressure of 419 mm. of Hg for two hours and then sacrificed. The lungs were kept in a 10 per cent formol solution for subsequent anatomical study. As control four other guinea pigs were killed at sea level and the lungs kept for a comparative study.

RESULTS OF THE VARIOUS INVESTIGATIONS. *Rate of ventilation, expired and alveolar air.* The results of these investigations are presented in table 1. They were made about thirty to forty minutes after exposure to a pressure of 419 mm. of Hg. Cases 2 and 3 showed a slight increase in the

minute volume of breathing, about a liter and five hundred cubic centimeters respectively, as compared with the control period, while in case 1 a decrease of a liter was found. Changes in the rate and depth of the respirations were not appreciably significant in cases 2 and 3, but in case 1 the rate of respiration diminished and an increase in the tidal volume was observed.

In all subjects a decrease in the alveolar carbon dioxide and oxygen tensions occurred especially marked in the latter and of about the same degree in all instances. These alterations and the fact that the decrease in the oxygen tension was not as marked as one would expect from the low tension of this gas in the inspired air, suggest that some compensatory mechanism, involving alveolar ventilation and gaseous interchange, had already been brought into play.

TABLE 2

*Observations on the arterial blood at sea level and at a barometric pressure of 419 mm. Hg (16,400) feet**

	CASE 1 (N. L. K.)		CASE 2 (A. H.)	
	Sea level	Altitude	Sea level	Altitude
Red cell count, 10^6	5.53	6.50	5.48	6.40
Hb, grams per 100 cc.	14.39	15.46	14.90	15.47
Viscosity	4.7	5.9	5.3	6.5
CO_2 content, volumes per cent	49.97	44.74	43.71	42.02
O_2 content, volumes per cent	19.25	14.16	19.05	15.39
O_2 combining power, volumes per cent	19.29	20.73	19.97	20.74
O_2 saturation, per cent	99.7	68.3	95.3	74.2

* Blood taken 60 to 70 minutes after being subjected to this pressure.

Arterial blood. This was obtained only in cases 1 and 2, and the results of the various determinations are given in table 2. An increase of about a million red cells per cubic millimeter occurred in both instances, accompanied by a proportionally less marked increase in the hemoglobin. Therefore, at the low pressure the average red cell contained less hemoglobin (23.8 and 24.3 $\gamma\gamma$ hemoglobin) than at sea level (26.0 and 27.2 $\gamma\gamma$ hemoglobin).

The carbon dioxide content of the arterial blood decreased moderately in case 1 and very slightly in the other case. The most significant and marked alteration was observed in the oxygen saturation; in both cases there was a pronounced reduction in oxygen content. From the normal values of 99.7 and 95.3 per cent the saturation decreased to 68.3 and 74.0 per cent respectively, indicating that a marked degree of anoxemia had been attained.

Total pulmonary capacity and its subdivisions. The results of these observations are presented in table 3. We wish to emphasize the constancy with which certain changes were observed in these three subjects. There was a diminution in the vital capacity, varying from 160 to 730 cc., and a relatively greater increase in the residual air, varying from 400 to 820 cc. The total capacity remained approximately the same in two subjects, while in one (case 2) a moderate increase occurred. The above changes were consequently reflected in the relative values (total capacity = 100 per cent). The ratios of vital capacity and residual air to total capacity decreased and increased respectively, but it is worth mentioning that in all

TABLE 3

*Determinations of the total pulmonary capacity and its subdivisions at sea level and at a barometric pressure of 419 mm. Hg (16,400 feet)**

	CASE 1 (N. L. K.)		CASE 2 (A. H.)		CASE 3 (W. S. MC.)	
	Sea level	Altitude	Sea level	Altitude	Sea level	Altitude
Absolute values						
Total capacity (liters)	6.53	6.62	5.13	5.52	5.28	5.37
Vital capacity (liters)	5.15	4.42	3.82	3.66	4.07	3.76
Mid capacity (liters)	3.26	3.68	1.61	2.30	1.85	1.85
Complementary air (liters)	3.27	2.94	3.52	3.22	3.43	3.52
Reserve air (liters)	1.88	1.48	0.30	0.44	0.64	0.24
Residual air (liters)	1.38	2.20	1.31	1.86	1.21	1.61
Relative values (total capacity = 100 per cent)						
Vital capacity (per cent)	78.9	66.8	74.4	66.3	77.1	70.0
Mid capacity (per cent)	49.7	55.6	31.4	44.6	35.0	34.4
Complementary air (per cent)	50.3	44.4	68.6	58.4	65.0	65.6
Reserve air (per cent)	28.8	22.3	5.8	7.9	12.1	4.5
Residual air (per cent)	21.1	33.2	25.6	33.7	22.9	30.0

* Determinations made between 50 to 80 minutes after being subjected to this low pressure.

cases the observed alterations in these ratios were within the limits of normality. The mid-capacity was at a higher level in two instances, and remained unchanged in the third.

The diminution in the vital capacity was variously reflected in its components; the complementary and reserve air were lower in two instances and slightly higher in the third. The alterations just described in the total pulmonary capacity and its subdivisions during the anoxemia induced by a low barometric pressure, indicate the development of a moderate degree of emphysema.

The respiratory dead space was calculated from the tidal volume and the

carbon dioxide and oxygen percentages of the alveolar and expired air. The results are presented in table 4. A moderate decrease was observed in two instances, while in subject 1 an increase was found. In this case there was also a large tidal volume which may possibly explain the increase in dead space, as it has been found by several observers that the higher the tidal volume the greater the respiratory dead space. The results of these investigations indicate that no significant alteration occurs in the volume of the respiratory dead space at a low barometric pressure.

Anatomical studies of the lungs of guinea pigs. The lungs of the guinea pigs exposed to the low barometric pressure for two hours presented uniform alterations, as compared with those remaining at sea level. The congested appearance of the lungs (fig. 1) was very pronounced and easily visible. On microscopic examination the findings were quite striking. There was observed a dilatation of the capillaries resulting in a thickening of the alveolar wall, in some places this was so marked as to encroach on

TABLE 4

The "respiratory dead space" at sea level and at a barometric pressure of 419 mm. Hg (16,400 feet)*

	CASE 1 (N. L. K.)		CASE 2 (A. H.)		CASE 3 (W. S. MC.)	
	Sea level	Altitude	Sea level	Altitude	Sea level	Altitude
Dead space from CO_2 percentages (cc.)	169	181	198	160	231	185
Dead space from O_2 percentages (cc.)	144	174	208	193	219	170

* Calculated from the CO_2 and O_2 percentages of the tidal and alveolar air and the tidal volume.

the alveolar space (fig. 2). This was particularly evident in the central parts of the lungs, while in the sections made through the peripheral zones there was less congestion and more dilatation of the alveoli (fig. 3). The changes just mentioned were uniformly present in all four animals, although their intensity varied somewhat in the different sections studied.

Discussion. The literature dealing with the adaptative changes which occur under the influence of a low oxygen tension is quite extensive and we can only mention those observations which have a direct bearing on the present investigations. An increase in the pulmonary ventilation with a consequent rise in the alveolar oxygen tension, and a fall in that of carbon dioxide, have been observed by many investigators (4), (5), (6), (7), (8), (9), (10), and regarded as the most fundamental adaptative processes. Our findings from this point of view are merely confirmatory in character and need no further discussion. The demonstration of a decrease in the oxygen saturation with a diminished carbon dioxide content in the

arterial blood was first made by the Cerro de Pasco Expedition (8) of Barcroft and his co-workers and has been later confirmed by other investigators (11), (12), (10). These changes are also present in the natives who are fully adapted to the abnormal environmental condition. Again our observations of the arterial blood corroborate these previous studies. The decrease in vital capacity following exposure to a low barometric pressure is an observation amply confirmed by many investigators. The related literature has been recently summarized by Schneider (13), (14). The decrease seems to be proportional to the lowering of the barometric pres-

Fig. 1. Gross appearance of the lungs of a guinea pig (upper) exposed for two hours to a barometric pressure of 419 mm. Hg in a low pressure chamber, as compared with those of a guinea pig at sea level (lower).

sure (11), (15), and its occurrence has been attributed to the engorgement of the alveolar capillaries and the resultant decrease in the elasticity of the alveolar walls (13), (14), (11), (15), a mechanism which has been previously suggested as an explanation of the decrease of the vital capacity in cardiac failure (16), (17).

Functional and structural changes in the chests and lungs of animals and men living at high altitude, or temporarily subjected to a low oxygen tension, have been frequently mentioned by many observers. Congestion and dilatation of the lung capillaries have been described by Kronecker (18)

in 1894, Tigerstedt (19) and Bartlett (20) in 1903, Jacobi (21) in 1914, Rippstein (22) in 1917, and in more recent years by Vacek (23), Campbell (24) and Schurbert (25). Spehl and Desguin (26) in 1909 made the in-

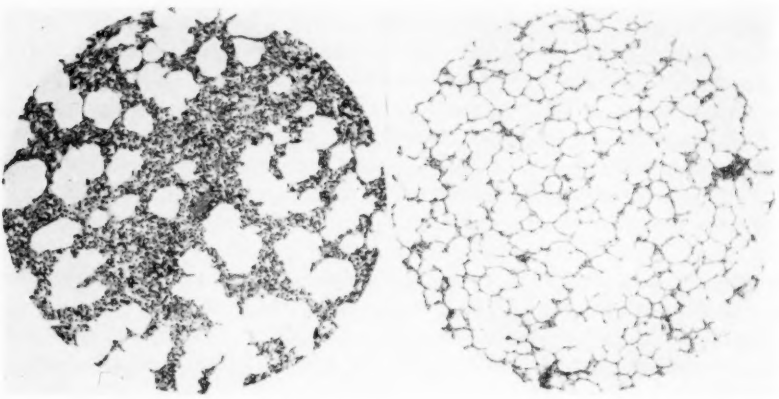

Fig. 2. Microscopic appearance of the lungs of a guinea pig (left) exposed for two hours to a barometric pressure of 419 mm. Hg in a low pressure chamber, as compared with those of a guinea pig at sea level (right). Section obtained from central zones—magnification × 50.

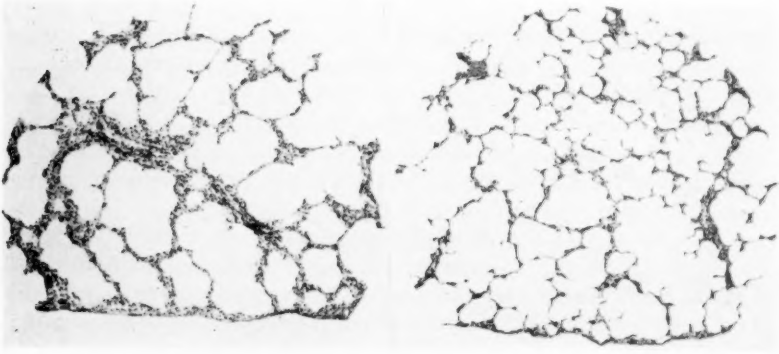

Fig. 3. Microscopic appearance of the peripheral zones of the lungs of a guinea pig (left) exposed for two hours to a low barometric pressure of 419 mm. Hg. On the right section of the lungs of a guinea pig at sea level—magnification × 50.

teresting observation that the lungs of rabbits living at 3,000 meters of altitude contain more blood than those at sea level.

Dilatation of the alveolar spaces, with an emphysematous appearance

of the lungs, chiefly at the peripheral zones, has been observed by several of the investigators just mentioned (23), (24). Prinzmental, Lonergan and Brill (27) found that pulmonary emphysema develops in dogs who are subjected to anoxemia. It is of great interest that analogous alterations in the lungs and chests have also been observed to exist in the natives of high altitude (28), (29), (30) and are associated with a high degree of physical adaptation to the low barometric pressure. Determinations of the residual air which were made in the members of the Cerro de Pasco Expedition (8) several weeks after their arrival, showed no changes from those made at sea level. The fact that these structural and functional changes occur within a short time after the exposure to the low barometric pressure, as shown in the present communication and that they are also present in those fully adapted to this unusual environmental condition suggests that they may play a part in the adaptative mechanism. This has been suggested by some of the investigators already mentioned (Rippstein, 1917; Jacobi, 1914; Vacek, 1925; Hurtado, 1932). The widening of the lung capillaries and the dilatation of the alveoli represent a condition favorable to a more efficient respiratory exchange, a process of prime importance in the adaptation to high altitude. This condition of emphysema with an enlargement of the vascular bed and a moderate increase in the residual air may then be interpreted as compensatory in nature, being perhaps one of the earliest adaptative mechanisms brought into play.

Similar occurrences have been observed in other conditions in which there is also an increased demand for respiratory function. Bohr (31) in 1907, and Rubow (32) shortly after, and Tamman and Bruns (33) in more recent years found a temporary distention of the lungs as a result of bodily exertion. This change has been interpreted as part of the respiratory adaptation to exercise (34), (35). The observations of Churchill (36) seem to afford the experimental proof for this assumption. This investigator showed that an increased demand for the oxygenation of the blood not only requires a higher rate of ventilation, but also an enlargement in the diffusing surface brought about by a widening of the capillary bed in the lungs. The importance of the latter mechanism was demonstrated by the observation that there occurred an increase in the oxygenation of the circulating blood, even in the absence of alterations in the rate and depth of breathing.

It is interesting to observe that there was slight evidence of an increase in the pulmonary ventilation in our cases in spite of the marked anoxemia as shown by the analysis of the arterial blood, indicating that the dilatation of the lung capillaries and higher volume of the residual air precede external changes in the respiration in effecting adaptation to low barometric pressures. This suggests that they may constitute one of the earliest adaptative mechanisms brought into play.

Whether the vascular changes in the lungs are produced by some me-

chanical factor depending on the low barometric pressure, or whether anoxemia plays the causative rôle is an interesting problem for investigation. The widening of the circulatory vessels is not restricted to the respiratory area; there is a generalized dilatation of the capillaries in the body, a fact observed by many investigators, and especially shown by Vanotti (37). The classical studies of Krogh (38) on capillary function have demonstrated that during increased activity a larger number of capillaries are open in the muscles, and that this general dilatation facilitates the free passage of blood flow and enlarges the diffusing surface for the respiratory gases. He has also demonstrated that during anoxemia the smaller arteries and capillaries in the rabbit's ear dilate as part of the compensatory mechanism. Henderson and Murray (39) in referring to Krogh's work interpret the adaptative mechanisms of the capillaries as being one of the major physiological activities in the body. The direct observations of the lung by Oklon and Joannides (40) and Wearn, Ernstene, Barr and German (41) have proved that there is an ample reserve capacity in the pulmonary vascular bed which may enlarge by an increase in the diameter of the capillaries and by opening of new channels temporarily closed.

The possible relation of anoxemia to capillary dilatation in the lungs may explain the important observation of Schneider (13) who found that the breathing of a high percentage of oxygen in the low pressure chamber or at high altitude prevents the decrease in the vital capacity or causes its return to a normal value.

It is possible that the dilatation of the alveoli, and the increase in residual air, are secondary to the capillary dilatation. Cloetta and Stänkle (42) observed that stagnation in the lesser circulation leads to an increase in the lung capacity. Binger (43) and Lundsgaard (44) attributed the early increase in the residual air found in cardiac patients to the vascular engorgement and the loss of elasticity of the alveolar wall.

The relation of these adaptative responses to the mechanism of mountain sickness has not yet been determined. It may be that mountain sickness depends simply upon an insufficiency of adaptive response, though the possibility remains that the adaptive changes described above, if carried to an extreme degree, might result in mountain sickness. This problem is worthy of further investigation.

SUMMARY AND CONCLUSIONS

Observations on the pulmonary ventilation, the composition of the alveolar and expired air, the carbon dioxide content and oxygen saturation of the arterial blood and the total pulmonary capacity and its subdivisions have been made in three healthy male subjects at a simulated altitude of 16,400 feet in a low pressure chamber. These investigations have been supplemented and correlated with anatomical studies of the lungs of guinea pigs exposed to the same experimental condition of a low oxygen tension.

The above observations, and a review of the literature, lead to the following conclusions:

1. The exposure to low barometric pressure caused the following changes in the arterial blood: an increase in the red cell count, hemoglobin and viscosity; a moderate decrease in the carbon dioxide content and a marked *diminution in the oxygen content and saturation.*

2. The ventilation per minute increased slightly in two subjects and was decreased in the remaining one. There occurred a marked reduction in the carbon dioxide and oxygen tensions of the alveolar air; the fall in the latter was not as marked as one would expect from the oxygen tension in the inspired air.

3. The respiratory response to the anoxemia included structural changes in the lungs, consisting chiefly of a widening in the capillaries and a dilatation of the alveoli. These changes were accompanied by a moderate increase in the residual air, and a corresponding decrease in vital capacity, with little or no change in the total pulmonary capacity. There appears to be sufficient evidence to suggest that these changes are compensatory in character. They tend to produce an increase in the surface for diffusion between the circulating blood and the alveolar air, a condition favorable for a more efficient exchange of the respiratory gases.

4. There was no significant alteration in the volume of the respiratory dead space at a low barometric pressure.

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THE EFFECT OF pH ON THE ABSORPTION OF SUGARS

ERNST GELLHORN AND LEON F. MOLDAVSKY

From the Department of Physiology, College of Medicine, University of Illinois, Chicago, Illinois

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In a former paper by Gellhorn and Skupa (1933) it was shown that the alteration in the composition of the perfusion fluid with regard to K and Ca had very marked effects on the absorption of glucose in a preparation in which the perfusion rate was kept constant. The results were interpreted as due to changes in the membrane characteristics of the gut. These observations raise the question whether it might be possible to alter the permeability of the gut by ions other than K and Ca. Therefore, experiments were undertaken in which the influence of pH on the absorption of sugars was studied since the profound influence of pH on cellular permeability (compare Gellhorn, 1929) and the extreme sensitivity of smooth muscle to changes in pH is well known (Fleisch, 1921; Atzler and Lehmann, 1921). We know, furthermore, that the gut muscle is also extremely sensitive to changes in pH as far as contractility and automaticity are concerned. There is, however, a principal difference between the mucosa- and the serosa-side of the gut since Magee and Southgate (1929) have shown that very small changes in the pH on the serosal surface have large effects, in contrast to small effects upon the mucosa.

The problem which was investigated in this paper involved four questions:

1. Does the change in pH affect the permeability of the gut to sugars within a range similar to that found effective in smooth muscle?

2. Are specific differences present between the effect of CO_2 and other acids?

3. Is the differential sensitivity of the mucosa and serosa reflected in permeability and is it possible to alter permeability from either side?

4. Does alteration in pH which influences glucose absorption show a similar effect on the absorption of xylose?

Fifty-two experiments were performed with a preparation described in previous papers (Gellhorn and Northup, 1932), in which the blood vessels supplying the gut and the gut itself were perfused separately. For the former we used Ringer's in the CO_2 experiments and ninety parts Ringer + 10 parts M/15 phosphate mixtures in all other experiments; the gut

was always perfused with isotonic sugar (glucose or xylose) solution. The rate of perfusion of the fluid in the blood vessels was kept constant. The sugar which permeated from the gut into the Ringer perfused blood vessels was estimated by the Folin-Wu method using a photo-electric colorimeter.

In addition, to meet the requirements of the particular problems investigated, it was found necessary to carry out the following:

1. Determination of pH, which was done in the earlier experiments by the Hastings-Sendroy colorimetric method and later by the quinhydrone electrometric method. The pH figures were checked in every case and the data presented represent averages of several determinations.

2. Determination of the percentage CO_2 in a gas sample used in aeration of the perfusing fluid was made with the Haldane apparatus. Here also duplicate determinations were made and the average presented.

The first group of experiments concerns the influence of the pH in the perfusate[1] on the absorption of glucose from the gut. Twelve experiments were carried out in order to investigate the influence of CO_2. The perfusion fluid was aerated with air containing CO_2 varying between 0.6 and 10 per cent. It was found, as the examples in table 1 indicate, that CO_2, if employed in a concentration somewhat higher than 0.6 per cent, increased reversibly the permeability of the gut to glucose. The pH determinations of the perfusate indicate that the least effective pH difference is about 0.05 pH. The effects were always very marked when the pH difference was 0.1 or more.[2]

In order to find out whether we are dealing here with a specific effect of CO_2 rather than pH, observations were made on the influence of phosphate buffered Ringer of varying pH on the absorption of glucose from the gut. Twenty-one experiments were carried out in which pH differences varying between 0.05 and 0.35 were studied on the acid and alkaline side. The results (table 2) show that on the acid side a pH difference of 0.1 and more distinctly increases the permeability of the gut to glucose; on the alkaline side, however, a pH difference of 0.1 did not show any effect (6 experiments), whereas a pH difference of 0.2 and 0.3 regularly led to an increase in permeability to glucose. The experiments indicate that the permeability of the gut to glucose is altered by changing the pH in the blood vessels to the acid or alkaline side. In either case the permeability is increased. It is found, however, that greater differences are necessary on the alkaline side than on the acid side in order to bring about increased glucose absorp-

[1] Perfusate = perfusion fluid in the blood vessels.

[2] The increased absorption of alcohol in the stomach and gut occurring in the presence of very high CO_2-concentrations (40–76 per cent), (Edkins and Murray, 1924–1926), is probably due to an alteration in circulation since CO_2 increases the diameter of the blood vessels.

tion. The effect of CO_2 and of a phosphate buffered solution is about the same if similar pH differences are involved. The great sensitivity of the serosa side of the gut to pH, as shown by Southgate's studies on automaticity, is reflected in a similar sensitivity of the gut in permeability to glucose, which is brought about by very small changes in pH.

In his studies on the permeation of cyanol from the blood vessels into the gut, Mond (1924) has already made some interesting observations indicating a differential sensitivity of the two sides of the gut to changes in pH. His studies, however, were only of a qualitative nature and since they deal with a dyestuff which is excreted in a direction opposite to that of

TABLE 1

The influence of CO_2 in the perfusate on glucose absorption

EXPERIMENT 1			EXPERIMENT 2		
Perfusion medium	Glucose, mgm. per cent	pH	Perfusion medium	Glucose, mgm. per cent	pH
O_2-Ringer	2.50	7.14	O_2-Ringer	2.50	7.15
O_2-Ringer	2.10	7.14	O_2-Ringer	2.00	7.15
1.9% CO_2-Ringer	4.00	6.80	1% CO_2-Ringer	4.00	7.10
1.9% CO_2-Ringer	4.05	6.70	O_2-Ringer	2.80	7.15
1.9% CO_2-Ringer	4.00	6.55	O_2-Ringer	2.50	7.15
O_2-Ringer	2.50	7.00	O_2-Ringer	2.30	7.15
O_2-Ringer	2.30	7.12			
O_2-Ringer	2.25	7.14			

TABLE 2

The influence of pH changes in the phosphate buffered perfusate upon glucose absorption

EXPERIMENT 3		EXPERIMENT 4	
Glucose, mgm. per cent	pH	Glucose, mgm. per cent	pH
3.60	7.14	2.50	7.20
3.40	7.14	2.50	7.20
3.30	7.14	2.50	7.20
4.30	6.96	3.30	7.40
4.18	6.96	3.30	7.40
4.18	6.96	2.80	7.40
2.30	7.14	2.50	7.20
2.50	7.14	2.50	7.20
		2.25	7.20

physiological absorption, the results are not quite comparable with ours. However, it may be mentioned that he found that extreme changes in pH at the mucosa side (pH 3.7) were necessary in order to bring about an increase in the cyanol excretion, whereas pH = 5.0 was effective on the blood vessel side. In order to further investigate these problems and to determine whether physiological changes in pH may affect absorption by changes in permeability, we changed the pH of the fluid with which the gut was perfused by using phosphate buffered solutions, whereas for the perfusion of the blood vessels Ringer solution was employed. It could be shown that comparatively small changes in pH (0.2 on the acid side, 0.7 on

the alkaline side) had very marked effects on the absorption of glucose (table 3). Here again it was found that increased acidity or alkalinity in the gut increased the permeability of the gut to glucose. The determination of the pH in the fluid with which the blood vessels were perfused indicated, however, that in these experiments the pH was altered not only in the gut but also in the blood vessels themselves. Therefore, the alterations in glucose permeability must be due either totally or partially to the alterations in the pH of the blood vessels.

It seems to be of theoretical, as well as practical, interest to know whether it is possible to alter the permeability of the gut by varying the pH in the gut itself. Under physiological conditions such alterations will never be accompanied by any marked changes in the pH of the blood on

TABLE 3

Influence of changes in pH in the buffered gut fluid upon glucose absorption

EXPERIMENT 5		
Glucose, mgm. per cent	pH of gut fluid	pH of perfusate
5.80	7.12	7.13
4.30	7.12	
11.30	6.70	6.72
7.80	6.70	
3.50	7.12	7.02
3.80	7.12	
3.30	7.12	

TABLE 4

Influence of changes in pH in the non-buffered gut fluid upon glucose absorption

EXPERIMENT 6			EXPERIMENT 7		
Glucose, mgm. per cent	pH of gut fluid	pH of perfusate	Glucose, mgm. per cent	pH of gut fluid	pH of perfusate
0.35	7.12	7.30	0.3	7.2	7.18
0.30	7.12		0.3	7.2	
1.20	8.08	7.34	1.0	5.87	7.22
1.00	8.08		1.1	5.87	
0.75	7.12	7.35	0.3	7.2	7.18
0.30	7.12		0.15	7.2	
0.30	7.12				

account of its great buffer capacity. In order to imitate physiological conditions in a more appropriate manner, we performed six experiments in which the pH in the gut was changed by the addition of acid or alkali without the use of any buffered solutions. The changes involved were pH 1.25 on the acid side and 1.0 on the alkaline side. The pH was determined also in each sample of the perfusion fluid obtained from the blood vessels. It was found that under these conditions the pH (table 4) in the blood vessels remained completely unchanged but that the absorption of sugar was increased. This increase was small compared with the effects obtained in the experiments reported above in which the results of changes in the pH on the serosa side of the gut were studied. Since the serosa side, as has been mentioned, remained unchanged, the effect must be attributed to changes in pH on the mucosa side.

We, therefore, come to the conclusion that changes in the pH of the perfusate, either to the acid or alkaline side, bring about an increase in sugar absorption from the gut, which, under conditions of a constant perfusion rate, must be due to an increase in the permeability of the gut to glucose. The permeability of the gut can also be altered from the mucosa side but considerably larger changes in pH are necessary and the effect is rather small.

It has been made probable by Wilbrandt and Laszt (1933), Lundsgaard (1933), and Wertheimer (1934), that in the absorption of glucose an intermediary esterification process takes place, which accounts for the greater absorption of glucose than that of xylose, for instance, as was found in

TABLE 5

Absorption of xylose under the influence of pH changes

A. pH ALTERED IN PHOSPHATE BUFFERED PERFUSATE—EXPERIMENT 8		B. pH ALTERED IN NONBUFFERED GUT FLUID—EXPERIMENT 9		
d-Xylose, mgm. per cent	pH of perfusate	d-Xylose, mgm. per cent	pH of gut fluid	pH of perfusate
2.80	7.03	1.00	7.00	
3.00	7.03	1.00	7.00	7.00
7.30	6.78	1.00	7.00	
7.50	6.78	1.30	5.00	6.98
4.00	7.03	1.40	5.00	
3.50	7.03	1.00	7.00	
2.00	7.03	0.30	7.00	7.02
		0.50	7.00	
		1.40	8.00	7.02
		0.30	7.00	7.04

Cori's well-known experiments (1925). It was therefore possible, since in our experiments glucose was at first exclusively used, that the changes in the ionic composition of the perfusates which altered glucose absorption and were interpreted as being due to changes in permeability may have been caused by the influence of these factors (in the experiments reported in this paper, for instance, of pH) on the esterification process. In order to decide this question, experiments were performed with xylose in which the effect of changes in the pH, either on the gut side or on the blood vessel side, was studied in regard to xylose absorption. As the examples given in table 5 show, the rules stated above in regard to glucose absorption hold equally well for xylose. This proves conclusively that the changes in

pH on glucose absorption under the conditions of our experiments are due to changes in the permeability of the gut.

The xylose experiments are interesting from still another point of view. It has been observed very frequently during this series of investigations, an observation already mentioned in one of the papers in this series, that in a considerable number of experiments the glucose concentration does not remain absolutely constant during the course of a two-hour experiment, but shows a rather regular decline. This was interpreted as being due to a gradually declining permeability of the gut since all the other conditions were kept constant. Taking into consideration the observations of Lundsgaard and others that in the absorption of glucose an esterification process is involved which partially accounts for the amount of glucose absorbed, it was possible that the decline in the glucose absorption occurring during the course of an experiment may be due to a decrease in the process of esterification. If this were the case, a decrease should not occur in xylose experiments. The examples reproduced in table 5 show, however, as well as other experiments not reproduced in the paper, that the same decline frequently occurred in xylose experiments. Therefore, the original interpretation is considered to be correct.

SUMMARY

In a preparation in which the gut and the blood vessels supplying the gut are perfused separately, the former with isotonic glucose solution, the latter with Ringer solution, and in which the circulatory rate was kept constant throughout the experiment, the influence of the pH on sugar absorption was studied.

It was found that:

1. Changes in pH to either the alkaline or acid side in the perfusion fluid of the blood vessels causes a reversible increase in glucose absorption. The threshold sensitivity lies at about 0.1 pH on the acid side and 0.2 on the alkaline side.

2. Increased acidity of the perfusion fluid due to CO_2 also increases glucose absorption. The range of pH sensitivity is similar to that observed in the experiments carried out with phosphate buffers.

3. If the gut is perfused with phosphate buffered glucose solutions small alterations in its pH bring about changes in glucose absorption similar to those described under 1. They are due to changes in the pH on the blood vessel side, which are regularly observed in these experiments. If, however, the gut is perfused with unbuffered solutions of various pH, comparatively large changes in pH (differences of one unit) bring about slight changes in sugar absorption, although the pH in the perfusion fluid of the blood vessels remains unchanged.

4. Experiments carried out with xylose absorption under similar con-

ditions lead to the same results. This indicates that changes in pH bring about alterations in gut permeability which are not due to interference with intermediary chemical processes involved in the glucose absorption.

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THE EFFECT OF FLUORINE ON CALCIUM AND PHOSPHORUS METABOLISM IN ALBINO RATS

EDITH M. LANTZ AND MARGARET CAMMACK SMITH

From the Department of Nutrition, Agricultural Experiment Station, University of Arizona, Tucson

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This laboratory has been interested in the study of fluorine because of its relation to the defect of human teeth known as "mottled enamel." As a part of this work, investigation has been made of the effect of fluorine upon calcium and phosphorus metabolism.

Numerous reports (1–9) in the literature on the appearance, composition, strength and histological structure of teeth and bones of fluorine fed animals suggest that the damaging effect of fluorine is due to interference with normal calcification. Few reports, however, have been made of the effect of fluorine on the balance between the intake and output of calcium and phosphorus. Forbes (10) compared the supplemental value of various calcium compounds including rock phosphate, in the feeding of swine, by determining the effect of these compounds upon the balance of calcium and phosphorus. A decrease in the retention of both calcium and phosphorus was reported for the rock phosphate fed swine. At this time there is reason to believe that the explanation of this finding is due to the presence of fluorine in the rock phosphate (3, 4, 11).

McClure and Mitchell (3) investigated the effect of the feeding of sodium and calcium fluoride upon calcium metabolism in albino rats. In this investigation they included calcium balance studies. The "paired feeding" method was used, the control rat being restricted to the same food intake as its fluorine fed littermate. Sodium fluoride was incorporated in the ration at levels of 0.0106 per cent, 0.0313 per cent and 0.0623 per cent fluorine and calcium fluoride at levels of 0.0313 per cent and 0.0623 per cent fluorine. Two ten day balances were determined on each pair of rats, the first during the first six weeks and the second during the last four weeks of a 10 week experimental period. Urine and feces were analyzed separately. They found that calcium retention was not affected by sodium or calcium fluoride at levels of 0.0106 per cent or 0.0313 per cent fluorine, but that it was lowered when either was fed at levels of 0.0623 per cent fluorine. In another paper (12) they reported that calcium fluoride fed to swine at a level of 0.017 per cent to 0.026 per cent fluorine had no defi-

nite effect on calcium retention. They did not include a study of the effect of fluorine on phosphorus metabolism in either of these papers.

It is the purpose of this paper to report the effect of feeding sodium fluoride on the calcium and phosphorus metabolism of albino rats of different ages.

Experimental materials and methods. Male albino rats from our stock colony, which is raised on Sherman's diet B (13), were used in all these balance experiments. Food records were kept and the excreta collected for each rat over a series of experimental periods of four or eight days in length. Distilled water was supplied ad libitum in bottles clamped to the outside of the cage.

Sherman's diet B was used as the control diet and the experimental ration consisted of the same diet to which sodium fluoride was added at 0.05 and 0.1 percentage levels. The food intake was not limited so as to exclude a possible starvation factor but a careful record was kept of all food consumed. Scattering of food was reduced to a minimum by the use of food jars with two inch flanges and rats which scattered food in spite of this precaution were not used.

In most cases, urine and feces were collected and analyzed separately although in a few preliminary experiments this was not done. The urine was absorbed on filter paper which was placed under the cages and changed daily. The filter paper was then washed repeatedly with small amounts of distilled water to which HCl had been added. It was found that washing was more efficient and filtering more rapid if the acidified wash water was hot. The washing was continued until the washings were colorless. From 1500 to 1800 cc. were usually used in washing the filter paper, pan and cage at the end of the balance period. The filtered washings were then made up to a volume of 2000 cc.

The feces (or combined urine and feces in the first few balances) were dried after the addition of a little HCl and alcohol, and then ashed in an electric muffle furnace at a temperature between 800°C. and 900°C. It was found that the ash dissolved more readily when the temperature was kept low. The ash was dissolved in a little 1-1 HCl and the solution made to a known volume with distilled water.

Calcium was precipitated as the oxalate in aliquots of the urine and ash solutions by McCrudden's method (14, 15) and titrated with standard potassium permanganate ($KMNO_4$). Phosphorus was determined colorimetrically by Fiske and Subbarow's method (16) in which ammonium-phosphomolybdate is reduced to a blue compound by amino-naphthol-sulphonic acid.

The calcium and phosphorus content of the food was determined for each lot of diet by the same methods.

In a preliminary experiment an attempt was made to study the effect

of fluorine upon the calcium and phosphorus balances of adult rats. The adult rats were taken from the stock colony and given the stock colony diet to which 0.1 per cent sodium fluoride had been added, while their litter mate controls were continued on the same diet without the addition of fluorine. The animals which had been transferred from the stock diet to the one containing 0.1 per cent sodium fluoride lost weight very rapidly and scattered food badly so that these balances were discarded.

The first successful balances were made on adult rats taken from the stock colony and given a ration to which 0.05 per cent sodium fluoride had been added. The balance between the intake and output of calcium and phosphorus was determined for these animals only when it was certain

TABLE 1

The effect of sodium fluoride on the calcium and phosphorus retention of adult rats

RAT NUMBER	WEIGHT	DIET	CALCIUM			PHOSPHORUS		
			Intake	Output	Retention	Intake	Output	Retention
			Mgm. per day	Mgm. per day	Mgm. per gm. per day	Mgm. per day	Mgm. per day	Mgm. per gm. per day
	gms.							
7246	455 to 460	Control	98.6	64.1	+0.075	95.8	76.6	+0.042
7272	455 to 458	Fluorine	84.8	54.5	+0.066	81.1	79.3	+0.021
7230	402 to 400	Control	94.8	62.9	+0.080	92.1	98.0	−0.015
7242	400 to 398	Fluorine	81.0	56.6	+0.061	74.3	87.6	−0.033
7246	460 to 464	Control	93.8	56.6	+0.081	91.1	88.5	+0.005
7272	458 to 460	Fluorine	88.9	62.2	+0.058	82.3	81.7	+0.001
7246	464 to 458	Control	88.2	61.9	+0.057	85.6	90.1	−0.010
7272	460 to 465	Fluorine	90.0	59.9	+0.065	86.1	99.0	−0.03
7240	466 to 466	Control	84.5	54.7	+0.064	82.1	77.5	+0.010
7272	465 to 468	Fluorine	70.8	47.1	+0.051	67.7	51.2	+0.035
7246	468 to 468	Control	85.5	68.2	+0.037	83.3	81.2	+0.004
7242	402 to 410	Fluorine	76.8	50.0	+0.066	75.9	77.0	−0.003

that they were maintaining their weight. Animals of the same age on the non-fluorine diet were used as controls.

As adult animals, these rats should normally be in calcium and phosphorus equilibrium. That this is the case with these animals was indicated by the very small retentions of calcium and phosphorus as shown in table 1. However, it may be noted that the fluorine-fed animals retained even less calcium than the controls and were frequently in negative phosphorus balance.

In a second series of experiments, the effect of fluorine upon the ability of young growing rats to retain calcium and phosphorus was investigated. Many calcium and phosphorus balances were carried on with growing rats

TABLE 2

The effect of sodium fluoride on calcium and phosphorus metabolism of growing rats of different ages

RAT NUMBER	PER CENT Na F ADDED TO RATION	CALCIUM RETENTION			PHOSPHORUS RETENTION				PER CENT OF Ca INTAKE IN FECES	PER CENT OF P INTAKE IN FECES	RATIO OF Ca TO P IN FECES
		Total, mgm.	Per cent of intake	Mgm. per gm. rat per day	Total, mgm.	Per cent of intake	Mgm. per gm. rat per day	Ratio of Ca to P retained			
Period 1. Age 28 to 36 days											
9416	0.0	164.2	64.1	0.2810	163.3	43.6	0.2796	1.006	Urine and feces not separated		
9413	0.05	83.4	50.3	0.1737	76.7	31.6	0.1598	1.087			
9415	0.05	147.7	53.4	0.2637	138.6	40.7	0.2475	1.065			
9412	0.1	68.2	44.8	0.1397	33.0	14.8	0.0676	2.006			
Period 2. Age 36 to 44 days											
10212	0.0	159.0	75.4	0.2390	152.3	45.8	0.2292	1.044	6.8	13.2	0.327
9416	0.0	282.0	76.5	0.3356	240.0	44.5	0.2857	1.175	Urine and feces not separated		
9413	0.05	157.9	60.4	0.2377	153.2	40.1	0.2307	1.018	8.1	10.7	0.481
10209	0.05	144.5	72.7	0.2271	142.0	45.3	0.2233	1.031	Urine and feces not separated		
9719	0.05	204.3	70.5	0.2605	142.7	46.2	0.1820	1.431	15.2	14.6	0.977
9412	0.1	41.2	23.5	0.0767	57.5	21.4	0.1072	0.716	Urine and feces not separated		
10206	0.1	55.0	38.3	0.1118	73.2	32.3	0.1489	0.540	36.7	24.1	0.963
9721	0.1	40.9	30.4	0.0730	75.7	46.3	0.1351	0.751	41.9	28.2	0.939
Period 3. Age 44 to 52 days											
10212	0.0	190.0	75.2	0.2360	165.5	41.4	0.2190	1.148	10.6	14.7	0.456
9416	0.0	299.3	77.0	0.2634	251.1	44.2	0.2210	1.192	17.7	19.2	0.629
9413	0.05	199.4	70.3	0.2157	183.4	45.3	0.2047	1.269	10.9	15.2	0.461
9415	0.05	200.8	62.8	0.1961	190.0	40.6	0.1855	1.059	23.1	19.0	0.832
10209	0.05	169.4	74.9	0.1604	133.4	38.1	0.1263	1.057	24.2	18.2	0.912
9412	0.1	71.3	36.7	0.1061	102.5	36.1	0.1673	0.682	37.1	23.6	0.995
10206	0.1	72.5	40.9	0.1195	106.3	38.0	0.1897	0.696	51.6	31.1	1.134
Period 4. Age 52 to 60 days											
9416	0.0	233.6	58.0	0.1219	239.5	40.7	0.1663	1.731	9.3	20.4	0.325
10212	0.0	204.1	77.8	0.1848	134.9	36.7	0.1222	1.298	34.3	18.5	1.270
9415	0.05	225.8	69.1	0.1511	194.8	40.8	0.1531	1.420	27.3	15.3	1.223
10206	0.1	66.7	43.7	0.0981	66.8	27.3	0.0982	0.999	24.7	24.6	0.638
9412	0.1	63.0	34.2	0.1080	73.0	27.1	0.0930	0.863	54.6	34.3	1.088

TABLE 2—*Concluded*

RAT NUMBER	PER CENT Na F ADDED TO RATION	CALCIUM RETENTION			PHOSPHORUS RETENTION				PER CENT OF Ca INTAKE IN FECES	PER CENT OF P INTAKE IN FECES	RATIO OF Ca TO P IN FECES
		Total, mgm.	Per cent of intake	Mgm. per gm. rat per day	Total, mgm.	Per cent of intake	Mgm. per gm. rat per day	Ratio of Ca to P retained			
Period 5. Age 60 to 68 days											
9724	0.0	138.2	47.7	0.1107	101.5	27.9	0.0813	1.362	37.5	22.7	1.320
9416	0.0	217.3	56.9	0.1281	208.5	37.3	0.1229	1.042	29.1	22.7	0.877
9413	0.05	198.5	64.8	0.1468	206.1	45.9	0.1525	0.963	29.3	13.3	1.500
9415	0.05	252.3	78.5	0.1659	179.3	38.1	0.1180	1.596	20.6	17.6	0.928
9719	0.05	247.8	69.4	0.1656	155.2	34.7	0.1038	1.407	16.4	13.5	0.830
9412	0.1	59.7	33.6	0.0697	68.4	26.3	0.0799	0.873	52.9	28.0	1.291
9421	0.1	41.0	36.7	0.0524	43.8	23.2	0.0610	0.936	36.5	33.4	0.880
Period 6. Age 68 to 76 days											
9416	0.0	174.8	51.2	0.0938	123.9	24.8	0.0690	1.411	41.7	29.2	0.987
9413	0.05	219.0	70.3	0.1456	138.2	30.3	0.0919	1.585	23.1	14.8	1.068
9415	0.05	241.0	71.9	0.1408	186.9	38.1	0.1091	1.289	20.7	15.1	0.937
9412	0.1	94.3	46.9	0.1025	85.6	29.1	0.0930	0.986	63.2	26.1	1.240
Period 7. Age 76 to 84 days											
10212	0.0	167.5	61.7	0.1150	103.4	25.8	0.0710	1.619	25.4	22.8	0.754
9416	0.0	141.7	47.5	0.0711	122.1	28.0	0.0613	1.160	41.5	28.9	0.984
9413	0.05	187.4	62.2	0.1126	179.1	40.6	0.1076	1.046	28.2	20.4	0.943
9415	0.05	194.3	64.4	0.1061	129.3	29.3	0.0706	2.429	20.1	17.1	0.873
10209	0.05	195.1	47.8	0.1257	80.3	20.9	0.0517	1.503	26.4	16.9	1.065
9412	0.1	45.7	27.8	0.0472	41.5	17.6	0.0429	1.101	58.5	31.8	1.282
10206	0.1	132.2	59.4	0.1238	98.2	32.7	0.0916	1.348	27.1	21.3	0.947
Period 8. Age 84 to 92 days											
9416	0.0	108.7	38.6	0.0509	117.4	28.5	0.0550	0.926	50.0	32.5	1.050
9413	0.05	171.1	63.8	0.0938	128.0	32.7	0.0702	1.336	31.3	19.3	1.108
9415	0.05	192.4	63.8	0.1002	121.2	27.5	0.0631	1.587	29.2	18.8	1.064
9412	0.1	75.2	38.6	0.0726	70.1	26.0	0.0679	0.926	54.2	31.9	1.160

of ages varying from 28 to 156 days. All of these animals had been fed the experimental diets since weaning. Eight consecutive balances of eight days each were made on one litter of four rats (nos. 9412–9416) during the period of most rapid growth, from 28 to 92 days of age. These results were supplemented with two to five balances of eight days on each of six other litters.

The effect of the feeding of sodium fluoride upon the metabolism of calcium and phosphorus is shown in table 2. A study of this table shows

that when 0.1 per cent sodium fluoride is added to the diets of young rats there is a decided decrease in their ability to retain both calcium and phosphorus. This decrease is apparent whether the retention is expressed as total milligrams of calcium or phosphorus retained, as per cent of the intake of calcium or phosphorus which was retained or as the milligrams of calcium or phosphorus which were retained per gram of rat.

For example, control rat 10212 in the experimental period between the ages of 36 to 44 days retained 159 mgm. of calcium and 152 mgm. of phosphorus, while his litter mate, no. 10206, which was given 0.1 per cent sodium fluoride, incorporated in the same basal ration, retained only 55 and 73 mgm. of calcium and phosphorus respectively. Expressed in another way the fluoride fed animal retained only 38 per cent of the calcium and 32 per cent of the phosphorus of the total intake whereas the litter mate which received no fluorine retained 75 per cent and 45 per cent respectively of the calcium and phosphorus in its food. The fluorine fed animal did not grow as rapidly as the control animal but expressed on the body-weight basis, the same difference in retention of calcium and phosphorus was evident. The non-fluorine fed control animal retained 0.2390 mgm. of calcium per gram of body weight whereas the fluorine fed animal retained only 0.1118 mgm. of calcium per gram of rat.

In general, it may be seen that during the period of active growth (28 to 52 days of age) the rats which received 0.1 per cent sodium fluoride in the ration retained approximately 35 per cent less of the calcium in their food intake than did their litter mates fed the same basal ration without sodium fluoride. Expressed on the basis of body weight the fluorine-fed animals retained less than half as much calcium as their normal controls of the same age.

The fluorine fed animals were stunted in growth and the decrease of the growth rate was of about the same magnitude as the decrease in the ability to retain calcium and phosphorus. The animals on the ration containing 0.1 per cent sodium fluoride present the short, stocky stature typical of calcium stunting. As these animals become older and heavier, marked bowing of the legs such as occurs in rickets results. See figure 1.

As the animals became older the difference in the ability of the two groups of rats to retain calcium and phosphorus disappeared. It is to be expected that when the rate of growth slackens there will be a decrease in the retention of both minerals in normal animals. In the control animals of this series the drop in the amount of calcium and phosphorus retained appeared rather suddenly when the animals were about 60 days of age. For example, during the first three balance periods, the amount of calcium retained per milligram of body weight for these rats ranged from 0.2360 to 0.3356 mgm. per gram of body weight. In the same period, the phosphorus retention dropped from more than 0.2 mgm. to about 0.15 mgm.

per gram of body weight. This finding that the retention of calcium and phosphorus decreases with age in the normal animal may be explained by the work of Hammett (16) who showed that there is an abrupt decrease in bone growth at 65 to 75 days of age.

The decline in the retention of calcium and phosphorus with age was less rapid in the rats fed the ration to which 0.1 per cent sodium fluoride had been added. In the 4th balance period the fluorine fed rats retained practically the same amount of calcium and phosphorus per unit of body

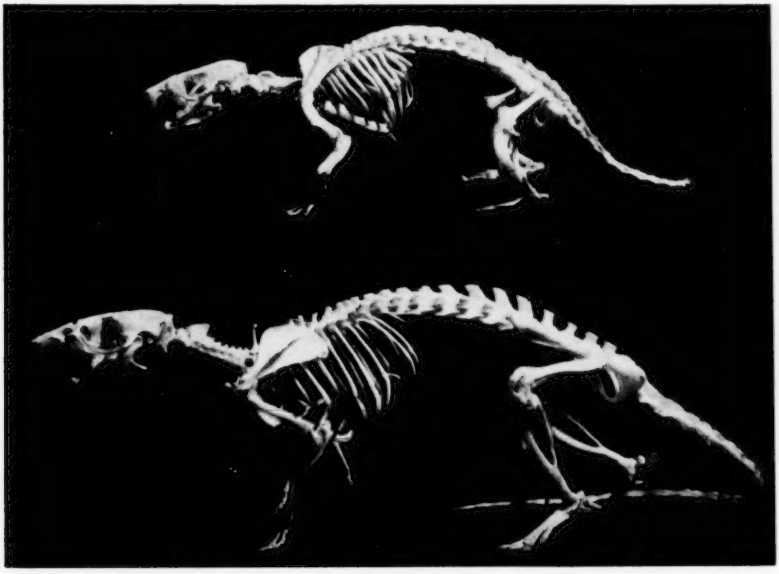

Fig. 1. The upper photograph is that of a rat fed a ration in which 0.1 per cent sodium fluoride was incorporated. The lower photograph is that of a litter mate fed the same basal ration without the addition of sodium fluoride. Note the stunted and deformed bone development of the fluorine-fed animal.

weight as they did in the first. For this reason when fluorine-fed animals are compared with non-fluorine-fed controls of the same age, the older animals show less difference in their ability to retain calcium and phosphorus.

It is interesting to note that by the time the fluorine-fed animals were 92 days of age, they were actually retaining a greater percentage of the calcium and phosphorus of their food and a greater amount of these bone forming elements per unit of body weight than the control animals. In some cases there was also a greater absolute retention of calcium and phos-

phorus in the fluorine-fed animals. It would appear from these results that the calcification of bone proceeds at a slower rate and is extended over a longer period of time in the fluorine-fed animals.

The data in table 2 which summarizes eight consecutive balances on rats 9412 to 9416 not only show that fluorine affects the absolute retention of calcium and phosphorus but also changes the proportion of calcium to phosphorus which is retained. With one exception, the young rats fed 0.1 per cent sodium fluoride have low calcium to phosphorus retention ratios as compared with the controls. The one exception, which was in the first balance period, was probably due to the fact that this animal failed to gain during this period. The retention ratio of the control animal 9416 fell below 1.0 in only one of the eight periods with an average retention ratio of 1.15, while the retention ratio of the fluorine fed rat 9412 ranged from 0.69 to 1.1 (with the exception noted above) and averaged 0.88. The low calcium to phosphorus retention ratios of the fluorine-fed rats indicates a greater growth of muscle tissue than of bone.

As the fluorine-fed animals became older, the retention ratios gradually increased and became more like those of the control animals which remained at approximately 1.1 showing no consistent change with age.

All of the comparisons so far have been made between animals receiving the higher level of fluorine, namely, 0.1 per cent sodium fluoride and their littermate controls which received no added fluorine. When only 0.05 per cent sodium fluoride is added to the diet of young rats the retention of both calcium and phosphorus is decreased but the decrease is much less pronounced than when 0.1 per cent is fed. In many cases the metabolism of calcium and phosphorus in these animals is so much like that of the control animals that if it were not for the striking effect of 0.1 per cent sodium fluoride, the significance of the differences which are seen might be questioned. Fluorine feeding at this level causes only a slight stunting in the growth of the animal. It is interesting to note that the decrease in the retention of calcium and phosphorus which is so abrupt in the control animals is more gradual in the animals fed fluorine at 0.05 per cent level. This supports the conclusion reached from a study of the retention of animals fed 0.1 per cent sodium fluoride, namely, that in the fluorine-fed animal calcification is proceeding at a slower rate and hence is extended over a longer period of time than in the control animals.

A difference in the path of excretion of calcium and phosphorus is also seen in the two groups of animals during the period of rapid growth. The per cent of the total intake of calcium and phosphorus and the relative amounts of these minerals which were eliminated by way of the feces have been calculated and are included in table 2. The fluorine-fed animals excrete a greater percentage of the total intake of both calcium and phosphorus by way of the feces than do the control animals. The fluorine-fed

animals also consistently excrete more calcium in proportion to the amount of phosphorus by this path than the control animals. For example, 41.9 per cent of the calcium in the intake of fluorine-fed rat 10206 in period 2 appeared in the feces, whereas the feces of its litter mate control, no. 10212, contained only 6.8 per cent of the calcium of its food. Rat 10206 also excreted a greater proportion of calcium than phosphorus by this path as shown by a fecal calcium to phosphorus ratio of 0.939 as compared with a ratio of 0.327 in the control rat 10212.

Again it may be noted that these differences between the fluorine-fed animals and their controls tend to disappear in the latter balance periods as the rats become older.

Too little is definitely known of the factors influencing the path of excretion of calcium and phosphorus to warrant drawing of conclusions as to the significance of these findings. However, these results suggest that at least part of the inability of the fluorine-fed animal to retain calcium may be caused by the non-availability of the calcium, perhaps due to the formation of the relatively insoluble calcium fluoride.

SUMMARY AND CONCLUSIONS

The calcium and phosphorus metabolism of albino rats of various ages, fed a basal diet to which 0.05 or 0.1 per cent sodium fluoride had been added, has been compared by means of balance experiments with that of rats given the same diet without sodium fluoride.

Growing rats fed the diet containing 0.1 per cent added sodium fluoride retained much less calcium and less phosphorus than the control animals reared on the same ration without the addition of sodium fluoride. This difference was seen whether the results were expressed as the total amount of calcium and phosphorus which were retained by the two groups of animals, as the percentage of the intake of calcium and phosphorus which was retained or as the milligrams of these minerals retained per gram body weight of the animal.

The two groups of animals retained different proportions of calcium and phosphorus as well as different amounts of these elements. The young fluorine-fed animals consistently retained less calcium in proportion to the amount of phosphorus retained, than the control animals receiving no fluorine. This is clearly shown by the calcium to phosphorus retention ratios which averaged slightly above 1.0 for the control animals while retention ratios as low as 0.54 were found for the younger animals in the fluorine-fed group.

As the animals grew older the difference in the ability of the fluorine-fed animals as compared with the controls to retain calcium and phosphorus was less marked, due to an abrupt decrease in the retention of these elements by the control animals at about 60 days of age. At this age, the

normally growing control rat had apparently passed the period of most rapid growth and calcification. In the growth stunted fluorine-fed rat the period of calcification was extended over a longer period of time. As a result of this condition the calcium and phosphorus retention of the fluorine-fed animal, expressed on the body weight basis, equalled or exceeded that of the control in the older animals.

A difference in the path of excretion of calcium and phosphorus was also noted in the two groups of rats. The animals fed the fluorine containing ration excreted far more calcium and more phosphorus in the feces than the control animals. It was also noted that the fluorine-fed animals excreted more calcium in proportion to the phosphorus by this path. It is probable that fluorine affects the metabolism of calcium and phosphorus by interfering with the absorption of calcium.

When the ration contained only 0.05 per cent sodium fluoride, the same differences in calcium and phosphorus metabolism were noted but of a much smaller degree.

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INTERACTIONS OF GONAD STIMULATING HORMONES IN OVARIAN DEVELOPMENT[1, 2]

H. L. FEVOLD AND F. L. HISAW

From the Department of Zoology, University of Wisconsin

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We have presented data (Fevold et al., 1931) which show that the follicle stimulating and the luteinizing preparations from the anterior lobe produce much greater development of the ovaries of rats when injected together than could be accounted for on the basis of an additive effect of the results when they were injected separately. Subsequently Evans and collaborators (1932) found that similar results were obtained when the gonad stimulating substance of pregnancy urine was injected together with a preparation from the anterior lobe and postulated their "activation" theory which designated the urinary factor as the "activating" agent and the growth hormone of the pituitary as the probable substance which was activated. They found that preparations, which contained the growth hormone but which were "free from the gonad stimulating hormone," could be readily activated while gonad stimulating extracts free from growth hormone did not give the reaction. Leonard (1932), however, was able to produce the augmentation effect by using gonad stimulating preparations which were definitely free from the growth substance. Later Evans et al. (1933a and b) confirmed the work of Leonard but claimed that the agent which acts together with the urinary gonad stimulating substance to produce the augmentation effect is a "synergistic" factor separate from any of the known gonad stimulating hormones of the anterior lobe.

In the meantime we had separated the two anterior lobe gonad stimulating hormones quite completely (Fevold et al., 1933a) which made it possible to study more carefully the interaction of these two substances and also their interactions with the urinary product. Our earlier work was confirmed and it was shown that the urinary substance is not essential for this reaction (Fevold et al., 1933b). Leonard and Smith (1934) found that urinary follicle stimulating hormone "Prolan A" injected together with the gonad stimulating principle of pregnancy urine also produced augmented results both in normal and hypophysectomized rats.

[1] Assisted by grants from the Committee for Research on Problems of Sex of the National Research Council.

[2] The sheep pituitary powder used in this work has been kindly furnished us by the research laboratories of Squibb and Company and The Wilson Laboratories.

In the following report we are presenting the results of a detailed study of the interactions of these different substances on the ovaries of immature rats.

EXPERIMENTAL. *1. Methods of preparation of follicular stimulating (F. S. H.) and luteinizing (L. H.) hormones.* The methods of extraction and purification are the same as those previously described (Fevold et al., 1933a) but more exact control of the final steps was found to be necessary to effect an efficient separation of the F. S. H. and L. H. As a result it seems that we have now separated them quite completely. It is relatively easy to obtain the luteinizing powder free from the F. S. H. but the elimination of the last traces of luteinizing hormone from the follicular stimulating fraction is more difficult. However, the method here given does eliminate all but extremely small amounts. The procedure is briefly outlined as follows:

The dried sheep pituitary is extracted with 50 per cent aqueous pyridine and the extract is partially purified by 33 per cent acetone and the two hormones partially separated by benzoic acid as previously described. The soluble fraction after benzoic acid precipitation is precipitated with acetone (4–5 vol.) and the precipitate taken up in water (1½ cc. per gm. sheep pit. powder). The solution is carefully adjusted to pH 4.1 or 4.2, 1 per cent cresol (Merk) by volume added and allowed to stand in the refrigerator for 24 hours. The precipitate is centrifuged off and united with the 33 per cent acetone insoluble fractions. A few drops of phenolphthalin are added to the solution at pH 4.1 to 4.2 and $Ba(OH)_2$ solution added gradually to faint pink. The precipitate is centrifuged off and discarded while the solution is again carefully adjusted to pH 4.1 or 4.2 with H_2SO_4, 1 per cent cresol added, and again put in refrigerator for 24 hours. The precipitate is then centrifuged off and added to the other insoluble fractions. The solution may be stored as such in the icebox or it may be precipitated by alcohol or acetone and taken up in the desired amount of distilled water, adding 0.4 per cent cresol as preservative. This solution contains the F. S. H. and seems almost entirely free from L. H.

The luteinizing factor is recovered from all the insoluble residues by extraction with phosphate buffer solution pH 8.0 (3 cc. per gm. original powder) for 12 hours repeating the extraction once. The residue is removed each time by centrifuging at 3000 R.P.M. for 15 to 20 minutes. The turbid solutions thus obtained are then neutralized to pH 4.1 or 4.2 and after standing for a few hours the precipitate is centrifuged off, dried, and stored as a dry powder. Yield 35 to 40 mgm. per gram of original powder. This powder may be further purified but such procedure offers no advantage in the work to be reported.

2. Combined action of follicular stimulating (F. S. H.) and luteinizing (L. H.) hormones on the ovaries of rats. As has been stated in our previous publications the F. S. H., when injected alone in immature rats (23 days old) for three days and the animals killed at the end of five days, produces ovaries of moderate weight which are composed of medium sized follicles. The luteinizing factor when injected in a similar manner produces no macroscopic change in the ovaries, the weights of the ovaries do not increase, and the uterus and vagina remain infantile. If, however, these two substances are combined and injected, the increase in weight of the ovaries is much greater than can be accounted for by the actions of the two separate hormones and the ovaries are filled with corpora lutea in all stages of development.

Table 1[3] demonstrates that the presence of a very small amount of the luteinizing hormone combined with 1.5 mgm. F. S. H. powder has a decided effect both on the weight of the ovaries and also on the qualitative results. The ovaries, resulting from 0.02 mgm. L. H. united with 1.5 mgm. F. S. H. powder, have increased in weight approximately twice as much as those with the F. S. H. powder alone and qualitatively they are luteinized while the others contain no lutein tissue. It is also apparent that the physiological limit of augmentation with this amount of F. S. H. is reached when 2 mgm. of luteinizing powder are added and any additional amount causes no further increase.

The actual weights of the ovaries resulting from combined injections of F. S. H. and L. H. seem to be more related to the amount of F. S. H. which is administered than the L. H. (table 2). While the size of the ovaries is

TABLE 1

Effect on weight of ovaries of combining various amounts of luteinizing hormone with 1.5 mgm. follicular stimulating hormone

F. S. H.	WEIGHT OF OVARIES	L. H.	WEIGHT OF OVARIES ON COMBINATION
mgm.	*mgm.*	*mgm.*	*mgm.*
1.5	25	20.0	105
		10.0	99
25 mgm. is the average weight of the ovaries of all controls treated with 1.5 mgm. F. S. H.		5.0	87
		2.0	101
		1.0	51
		0.5	52
		0.2	62
		0.1	44
		0.02	38

by no means proportional to the amount of F. S. H. given, still the weight decreases steadily and regularly with decreasing dosage of F. S. H.

In table 3 a comparison is made of the weights of ovaries produced by combination of equivalent amounts of F. S. H. and L. H. with those produced by an equivalent amount of the unfractionated pyridine extract. The data show that, although some is undoubtedly lost in the separation and purification of the two substances, still the results are quite comparable. Microscopic examination shows that the ovaries are identical in

[3] In the tables the weights of the ovaries of rats treated with L. H. are not given since the weights of these are the same as those of immature untreated animals (av. 15 mgm.). The facts presented by the tables have been repeatedly demonstrated on large numbers of rats. The actual data presented however represent final experiments set up specifically to illustrate the paper and each value given represents the average weights obtained from 3 to 6 animals in each instance.

that they contain corpora lutea and follicles in all stages of luteinization. Neither of the two substances has been injured nor have their physiological properties changed in the process of separation since when they are recombined we again have a preparation similar to the original unfractionated extract. This demonstrates that the action of the unfractionated extract is due to the combined effects of two separate factors each of which produces its own individual and separate changes in the structures which are present in the ovaries.

TABLE 2

Effect on weight of ovaries of combining various amounts of follicular stimulating hormone with 10 mgm. of luteinizing hormone

F. S. H.	WEIGHT OF OVARIES	WEIGHT OF OVARIES ON COMBINATION
mgm.		
2.0	30	106
1.0	16	92
0.5	14	77
0.25	14	50
0.12	14	21

TABLE 3

Comparison of the effect produced with combined equivalent amounts of F. S. H. and L. H. with those produced with equivalent amounts of the original unfractionated pyridine extract

F. S. H.	WEIGHT OF OVARIES	L. H.	WEIGHT OF OVARIES ON COMBINATION	WEIGHT OF OVARIES ON EQUIVALENT AMOUNT OF UNFRACTIONATED EXTRACT
mgm.		*mgm.*		
2.0	33	20	111	
1.0	17	10	84	96
0.5	15	5	54	62
0.25	14	2	25	27

Incidentally it is of interest to note the sensitivity of rat ovaries to the luteinizing hormone and also to demonstrate the purity of the follicle stimulating preparation from L. H. by means of mathematical calculations. Two hundredths of a milligram (0.02 mgm.) of luteinizing powder, when combined with 1.5 mgm. follicle stimulating powder causes marked augmentation and luteinization of the ovaries (table 1) and since 1.5 mgm., or several times this amount of follicle stimulating powder, causes no luteinization alone it must contain less than 0.02 mgm. luteinizing powder. One and five-tenths milligram (1.5 mgm.) of the follicle stimulating preparation was originally associated with 15 mgm. luteinizing powder since these

quantities represent equal amounts of original pituitary powder (table 3). Consequently less than (0.02/15 × 100) 0.13 per cent of the original luteinizing activity remains in the follicle stimulating preparation or 99.8 per cent of the luteinizing factor has been separated from the follicle stimulating hormone.

3. Combined action of follicular stimulating hormone (F. S. H.) and the gonad stimulating principle from pregnancy urine (P. U.). The two anterior lobe hormones (F. S. H. and L. H.), when either is combined with the gonad stimulating principle from pregnancy urine, produce quite different results. This is of course what might be expected since P. U. is primarily a luteinizing hormone with relatively weak follicular stimulating ability. It is, therefore, more similar in action to the L. H. of the pituitary and should act synergistically with the follicular stimulating hormone. This has been found to be the case since L. H. does not augment the action

TABLE 4

Effect on the weight of the ovaries of combining various amounts of F. S. H. with a constant amount of P. U. hormone

F. S. H.	WEIGHT OF OVARIES	P. U.	WEIGHT OF OVARIES	WEIGHT OF OVARIES F. S. H. + P. U.
mgm.	*mgm.*	*mgm.*	*mgm.*	
3.0	38	4.6	27	150
2.0	28			111
1.0	18	27 mgm. is the average weight		135
0.5	16	of the ovaries of all rats		95
0.25	15	treated with 4.6 mgm. P. U.		68
0.12	14			62
0.01	14			42

of P. U. but when F. S. H. is combined with P. U. augmentation of the weight of the resulting ovaries is very marked.

Table 4 shows that only small amounts of the F. S. preparation are necessary to produce very marked augmentation. Thus it is seen that it takes approximately 1 milligram of F. S. powder to produce any noticeable increase in the weight of the ovaries but 1/100 of this amount or 10 gamma is sufficient to augment the effect of P. U. very markedly. One milligram of the F. S. preparation, therefore, contains at least 100 units for this reaction.

Evans and co-workers (1933a) believe that the substance which produces augmentation when combined with P. U. is a separate factor from other known gonad stimulating hormones. They state that the "synergistic" factor is shown to be different from the gonad stimulating substances in that these are destroyed by treatment with acetic or formic acid, whereas the "synergistic" factor is not. In a later paper (1933b) they state that this factor has a definite amount of gonad stimulating activity but that it

is different from the activity of a real gonad stimulating preparation in that its action is more rapid and is not sustained except by continued injection.

Since our purified preparations of the F. S. H. showed such strong potency for producing augmented results with P. U. as well as with L. H., we attempted to prove the presence of a second substance in addition to the F. S. hormone. We therefore treated the F. S. preparation with acetic acid as described by Evans. We found that such treatment not only reduced the follicular stimulating potentiality very markedly but the augmentation ability likewise decreased. In fact after 18 hours of such treatment, which is recommended, 1 mgm. of the same preparation, of

TABLE 5

P. U. hormone + constant amount of F. S. H.

F. S. H.	WEIGHT OF OVARIES	P. U.	WEIGHT OF OVARIES	WEIGHT OF OVARIES F. S. H. + P. U.
mgm.	*mgm.*	*mgm.*	*mgm.*	
1.0	17	9.0	38	130
		2.0	21	94
17 mgm. is the average weight		1.0	18	122
of the ovaries of all rats		0.4	15	40
treated with 1.0 mgm. F. S. H.		0.2	15	47
		0.1	15	20

TABLE 6

Time as a factor in the increase of ovarian weight when immature rats are treated with follicle stimulating hormone

	DOSAGE			
	0.5 mgm.	1 mgm.	2 mgm.	4 mgm.
Weight of ovaries 72 hrs. (mgm.).....	25	40	50	60
Weight of ovaries 120 hrs. (mgm.).....	15	24	23	32

which 0.01 mgm. was sufficient for augmentation previous to treatment, showed no activity whatever.

It was possible to greatly reduce the follicular stimulating activity by treatment with acetic acid for various periods so that a given dose which, previous to treatment would elicit macroscopic stimulation of the ovaries, no longer would do so but would show augmentation effects with P. U. However, this is also true previous to acid treatment and merely demonstrates the same relationship shown in table 4, namely, that it takes 100 times the dose which produces augmentation to produce macroscopic follicular growth. In all cases where the follicle stimulating ability was completely destroyed by the acid, augmentation ability was likewise absent.

The rapid response which is used as a criterion to differentiate the "synergistic" factor from gonad stimulating hormones is a characteristic of the follicle stimulating hormone previously described by us (Fevold et al., 1931, 1933a). The ovaries of rats injected for 60 hours with F. S. preparations and autopsied at 72 hours are heavier than those of rats similarly treated and autopsied at 120 hours (table 6). Most gonad stimulating preparations contain both the F. S. H. and L. H. factors, and when both are present the development of the ovaries continues for a longer time since luteinization takes place during and after follicular development and the ovaries attain their greatest weight only after 100 to 120 hours. When pure F. S. H. is used, however, only follicular development takes place and this is apparently completed sooner. This fact does not differentiate the synergistic factor of Evans from the F. S. H.

In further attempts to demonstrate the non-identity of the F. S. H. and the "synergistic" factor, we prepared the "synergistic" preparation according to the method outlined (Evans et al. 1933b). From 750 grams of fresh sheep pituitaries we obtained 3.8 grams of a crude powder which when injected in amounts of 5, 3, and 1 mgm. gave ovaries weighing 50, 48, and 25 mgm. respectively. For comparison we used a sample of synergistic powder from Doctor Evans' laboratory obtained through the courtesy of Dr. P. R. Austin. This powder, when injected in the same amounts as our own, produced ovaries weighing 44, 35, and 25 mgm. All of the ovaries in both cases were heavily luteinized. The two preparations therefore appear to be identical in gonad stimulating ability. A third sample of "synergistic" powder, prepared by Evans' method was obtained from Doctor Gustus of the Upjohn Company. This material was almost totally insoluble in water whereas the first two were quite readily emulsified giving opalescent solutions. It was also much less active but 20, 50 and 100 mgm. of this powder gave increasingly large luteinized ovaries weighing 40, 90 and 115 mgm. It is thus apparent that all three of these powders contain both F. S. H. and L. H.

When 3 mgm. of the "synergistic" powder, prepared in our laboratory, were injected with 1 mgm. of our F. S. powder ovaries weighing 87 mgm. were produced while the same amount injected with 5 mgm. luteinizing powder showed no increase over that elicited by the "synergistic" preparation alone, the ovaries weighing 49 mgm. Entirely similar results were obtained when comparable doses of the other two powders were injected with the F. S. H. and L. H. preparations. In this respect these three preparations are comparable to our crude unfractionated pyridine extract.

One gram of the crude "synergistic" powder was then emulsified in 100 cc. distilled water, according to their directions, and carefully adjusted to a pH of 4.4. The precipitate which separated out after standing 8 hours was dried and weighed. The yield was 0.32 gram. The soluble fraction

was precipitated, dried and weighed. Yield, 0.64 gram. This soluble powder is the active "synergistic" powder. The two preparations were injected separately and in combination with each other. The "synergistic" powder (pH 4.4 soluble) was also injected in combination with our luteinizing preparation while the insoluble (pH 4.4) powder was injected with our F. S. preparation. The results are presented in table 7.

As previously noted, the original crude "synergistic" preparation produced augmentation when combined with the F. S. powder but did not do so when combined with the luteinizing factor. However, after isoelectric precipitation the "synergistic" preparation shows decided augmentation when recombined with the isoelectric precipitate or with the luteinizing powder and does not do so with the F. S. preparation. The isoelectric precipitate on the other hand does produce augmented results with our

TABLE 7

Combination of crude and purified "synergistic" powder with follicle stimulating and luteinizing hormones

I. CRUDE "SYNERGISTIC" POWDER			II. PURIFIED "SYNERGISTIC" POWDER		
	Materials injected	Ovarian weights		Materials injected	Ovarian weights
		mgm.			*mgm.*
A	1 mgm. F. S. powder	18	A	1.5 mgm. F. S. powder	33
B	3 mgm. Syn. powder	48	B	1.5 mgm. Syn. powder	27
C	2 mgm. Lut. powder	14	C	5 mgm. pH 4.4 Insol.	16
	A + B	87		powder	
	B + C	49	D	2 mgm. Lut. powder	14
				A + B	48
				A + C	104
				B + C	133
				B + D	99

F. S. preparation and does not do so with the luteinizing powder. In other words, the purified "synergistic" preparation contains primarily the F. S. H. but does contain some L. H. since it does not give pure follicular growth. However, enough L. H. has been removed from the original powder so that what before had an excess of luteinizing hormone which caused augmentation when F. S. H. was added, now contains an excess of F. S. H. which can produce more ovarian growth with additional luteinizing substance. The fact that the purified "synergistic" preparation produces augmented results when combined with L. H. is also shown by table 2 of Evans' paper (1933b).

A morphological basis for an explanation of the augmentation reaction has been sought through a histological study of the ovaries of both normal and experimental animals. A careful count of the primary follicles,

(those having two or more layers of granulosa cells) and the antra-containing follicles indicates that the F. S. H. (or something associated with it) is the factor which produces primary follicles. The ovaries of rats which have been treated with F. S. H. preparations show a great increase in the number of primary follicles over those of normal untreated animals. On the other hand the luteinizing hormone decreases the number of primary follicles with a proportionate increase in antra-containing follicles and the total follicular count remains the same as in normal untreated animals.[4]

It appears therefore that development in the ovaries may take place as follows: the F. S. H. produces primary follicles, the L. H. acts on the primary follicles to develop antra, the F. S. H. then acts on the antra-containing follicles to produce macroscopic follicles after which the luteinizing hormone can again act to produce corpora lutea. It seems therefore to be a 1, 2, 3, 4, reaction in which the odd numbers designate the action of the F. S. H. while the even numbers designate the functions of the L. H.

These results offer a plausible explanation for the augmented results produced when the F. S. H. and L. H. are injected simultaneously. The normal immature ovary of a 23 day old rat contains follicles with and without antra. The F. S. H. can act on those which have antra, producing macroscopic follicles and it also causes an increase of primary follicles. When the luteinizing hormone is injected in an immature 23 day old rat it can only produce antra in the primary follicles already present which change is not readily measurable by ovarian weights. It cannot produce corpora lutea because the next link in the chain is missing; however, occasionally a corpus or two is seen in the ovaries of immature rats treated with luteinizing hormone alone. These come apparently from the occasional follicles which have developed past the small antra stage due to F. S. H. secreted by the animals own pituitary. A similar viewpoint also has been expressed just recently by Aschheim (1933).

The great growth produced when a mixture of the two hormones is injected is readily explained on this basis since all these changes are going on simultaneously. The small antra-containing follicles are changed to macroscopic follicles by F. S. H. then to corpora lutea by the L. H. More antra containing follicles are produced by the L. H. and the above process is repeated. During this time more primary follicles are laid down which then become available for the L. H. and F. S. H. to exert their effects. Consequently the size of the ovary produced is limited only by its physiological capacity to react and the proportionate balance of the two A. P. hormones.

The histological effects of the P. U. hormone from pregnancy urine have also been studied. While in this case the picture is complicated by the fact that this factor contains some follicular stimulating ability in addition

[4] The details of this phase of the work will be reported by Mr. C. E. Lane.

to its major luteinizing action, still the histological picture shows that its main effect is that of luteinization. It increases greatly the antra containing follicles in the ovary but also puts down some additional primary follicles. This being the case it is evident that it should give greater effects when combined with the F. S. H. and no additional response when combined with L. H. Its luteinizing power surpasses its follicle stimulating ability and more F. S. H. can therefore be utilized while addition of more L. H. does not supply what is lacking but merely adds to a surplus.

Discussion. The activation theory proposed by Evans had its chief foundation in the fact that it apparently explained the augmented results produced by P. U. plus a substance from the anterior lobe. However, in proposing his theory Evans did not take into account the work previously published from this laboratory showing that the same type of response was obtained by recombining two principles which had been separated from the anterior lobe itself. If this reaction is an activation as proposed by Evans, then the activation has already taken place in the unfractionated extract and we would have to assume that the reaction is completely reversible and that total reactivation takes place upon separation of the two factors.

It seems that the ultimate results of the effects of P. U. + F. S. H. and L. H. + F. S. H. on the ovaries of rats are the same in that large luteinized ovaries are produced. The mechanism within the ovary by which this development is attained may be somewhat different but we believe that in the main the process is the same. In the combination of F. S. H. with P. U. it would seem that the F. S. H. is the factor which produces follicular structures on which the urinary hormone can act and produce larger ovaries. This seems to be true since the amount of F. S. H. necessary to act with P. U. for augmentation is very small while the amount of P. U. in comparison cannot be reduced much below that which by itself will produce macroscopic development of the ovaries. In this respect the facts bear out the theory proposed by Hamburger (1933) who suggests that the A. P. gonad stimulating hormone sensitizes the ovary so that P. U. can demonstrate its effect. From our results it is apparent, especially when we are dealing with the luteinizing and follicular stimulating hormones, that the combined result of the two is an expression of their separate functions in the development of the ovary. The actual manner in which the P. U. hormone works is at present a little obscure since it seems to have some of the ability of the F. S. H. but much luteinizing ability.

From the results of our experimental work we do not think that it is necessary to postulate a third "synergistic" factor. We have not of course definitely eliminated the possibility that our preparation of the F. S. H. also contains such a factor, but we have not been able to obtain any evidence which indicates that the "synergistic" factor is anything but the follicular stimulating hormone. The reactions claimed as differentiating

the "synergistic" factor from the other known gonadotropic hormones demonstrates no difference whatever in our hands. This is contrary to the statement that "these facts clearly indicate that the synergistic factor, prepared in this laboratory cannot be identified with either the luteinizing or follicle stimulating fractions and is actually a third gonadotropic substance from the pituitary" (Evans et al., 1933b). Acetic acid is equally destructive to the follicle stimulating and the augmenting activity, the faster gonadotropic action of the "synergistic" factor is one of the characteristics of the F. S. H., and crude "synergistic" factor can be fractionated so that the purified "synergistic" preparation contains predominantly F. S. hormone while the fraction which is eliminated contains very little F. S. H. but much L. H. This is evident from the reactions of these two substances when recombined or when either of them is combined with our "pure" follicle stimulating preparation or with the luteinizing powder.

SUMMARY AND CONCLUSIONS

1. An improved method for the separation of the follicle stimulating and luteinizing hormones of the anterior lobe of the hypophysis is briefly outlined. The follicle stimulating preparation obtained by this method contains less than 0.2 per cent of the original luteinizing activity.

2. The F. S. H. and L. H. act together on the ovary to produc macroscopic follicles and corpora lutea. Both seem to have separate functions to perform in a 1, 2, 3, 4 sequence which must take place in the order designated. Each hormone is dependent on the previous action of the other on the ovary and consequently the combined effects of the two on the ovaries of rats as measured by the weight and appearance of the ovaries are greater than would be expected from the results obtained by injecting each separately in different animals. The effect of each hormone has been augmented by the action of the other.

3. It has been shown that P. U. acts in a manner analogous to the luteinizing hormone in the augmentation reaction.

4. The existence of a separate "synergistic" factor from the anterior pituitary could not be demonstrated. The F. S. H. and the "synergistic" factor could not be identified as two separate substances.

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THE CARDIAC OUTPUT IN MAN

An Adaptation of the Katharometer for the Rapid Determination of Ethyl Iodide in Estimations of Cardiac Output by the Ethyl Iodide Method. A Study of the Effect of Posture upon Cardiac Output and Other Circulatory and Respiratory Measurements

JOHN S. DONAL, JR., CLARENCE J. GAMBLE AND ROBERT SHAW

From the Laboratory of Pharmacology of the University of Pennsylvania

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The methods which have been developed for the measurement of the cardiac output in man have been applied by many physiologists to the study of changes in cardiac output occurring when the posture of the subject is altered. Their results have been conflicting. One group of investigators, comprising the majority of those who have studied the subject, finds that the output of the heart is less in the vertical than in the horizontal position. The others secure results which show no significant variation between the two. The striking differences between the various methods employed for the determination of the bloodflow in man suggest that these may be the cause of the lack of agreement of the results. This cannot be the sole source of the variations, however, for successive investigators, using substantially the same method, have come to opposing conclusions.

The results secured by previous investigators have been assembled in table 1. The ratios of the cardiac outputs in the standing to those in the lying position vary from approximately 1.00 found by Lindhard (1913) in males, and by Grollman (1928 and 1930) and by Schellong and Heinemeier (1933) in all subjects, to as low as 0.50 in the results of Field and Bock (1925). All observers agree that the output per beat is significantly decreased in the vertical positions. Reference to their publications shows, also, that all find the arterio-venous oxygen difference to be greater in the sitting and standing postures than when the subject is horizontal.

The determinations made with the method of Henderson and Haggard (14, 22, 7) have not been included in the table as this method neglected the ethyl iodide shown by subsequent investigators (24, 19, 32) to be present in the venous blood.

Since the method of Starr and Gamble (1928) differs in important elements from the others, it seemed worth while to extend the observations of Starr and Collins (1931) to a larger number of subjects. In this method, in

contrast to the others employing foreign gases, the measurement of the rate of absorption is not limited to a single circulation time. An additional advantage lies in the fact that in the calculation of the cardiac output neither the absorption of oxygen nor the excretion of carbon dioxide appears as a factor. This may be expected to make the results more independent of the changes in metabolism which are known to occur with changes in position.

TABLE 1

The ratios of the cardiac output in the sitting and standing to that in the lying position, as determined by various investigators

INVESTIGATOR	GAS EMPLOYED	NUMBER OF SUBJECTS	NUMBER OF EXPERIMENTS	CARDIAC OUTPUT PER MINUTE		STROKE VOLUME	
				Sitting ÷ lying	Standing ÷ lying	Sitting ÷ lying	Standing ÷ lying
Lindhard (1913) males	N_2O	4	39	1.06		0.99	
Lindhard (1913) females		3	19	0.86		0.78	
Collett and Liljestrand (1924)	N_2O	1	1	0.82	0.73	0.81	0.68
Field and Bock (1925)	CO_2	10	13	0.76		0.74	
		7	9		0.50		0.35
Lawrence, Hurxthal, and Bock (1927)	CO_2	10	10	0.87	0.67	0.85	0.52
Turner (1927)	CO_2	25	43	0.91		0.72	
		25	27		0.89		0.60
Grollman (1928)	N_2O	14	14	0.96	0.95	0.88	0.77
Grollman (1930)	CO_2	4	4	1.00	0.98	0.93	0.73
Grollman (1930)	O_2	4	4	0.96	1.01	0.93	0.76
Grollman (1930)	C_2H_2	4	4	1.00	0.96	0.94	0.73
Kroetz (1930)	C_2H_2			0.78		0.73	
Starr and Collins (1931)	C_2H_5I	4	8		0.90		
Fisher (1932)*	C_2H_2	2	7	0.84		0.79	
		10	52		0.82		0.76
Schellong and Heinemeier (1933)	C_2H_2	16	27		1.03		0.81
H.-E. Bock (1934)	C_2H_2	16	23		0.76		0.62
This investigation	C_2H_5I	11	11	0.87		0.76	
		21	23		0.82		0.60

* Averages of ratios in published experiments.

The results of this investigation showed a significant difference between the cardiac output in the various positions, when measured in normal fasting subjects at rest. The average output in the standing position was 82 per cent, and that in the sitting position 87 per cent of the output when the subject was lying on his back.

This progressive change with rotation of the body in the field of gravity suggested that the output might be still higher if the rotation were carried

beyond the horizontal. Bielschowsky (1932), using acetylene, found that the blood flow was unchanged in normal persons when the foot of the bed was raised on blocks to an unspecified angle. With persons whom he considered to have weak circulatory mechanisms he detected a moderate increase.

To test the effect of further inversion, the cardiac output of two normal subjects was measured in the vertical position with the head down. In both cases it was found to be less than when the subject was horizontal.

METHODS AND APPARATUS. A major difficulty in the applications of cardiac output methods lies in the fact that they have been exceedingly time consuming. Doubtless for this reason duplicate estimations, so essential for the proper interpretation of quantitative results, have seldom been made in cardiac output studies. Therefore, believing that greater rapidity without sacrifice of accuracy was essential to the proper advancement of the field, we set ourselves the task of developing a physical method of analysis.

The low thermal conductivity known to be a property of ethyl iodide gave the hope that its concentration in air might be estimated much more rapidly by the katharometer.[1] This apparatus was used by Lamson and Robbins (1928) for the determination of carbon tetrachloride. It has been described in detail by Palmer and Weaver (1924) and a good review of the designs and uses of the instrument has been given by Daynes (1933).

Modifications of the katharometer required for determination of ethyl iodide. The circuit diagram of the katharometer is shown in figure 1. In using the form of the instrument described by Ledig and Lyman (1927)[2] certain modifications were found necessary.

Large and random oscillations in the relative resistances of the two cell wires were encountered when the liquid in the bath was stirred mechanically. When the cells were immersed in light mineral oil in a sheet metal container, surrounded by a larger thermostatically controlled oil bath, these oscillations were abolished.

Liquid air was used to condense the ethyl iodide contained in that portion of the sample admitted to one of the cells.[3] To prevent the great alteration in thermal conductivity, which would result from condensation of water vapor and carbon dioxide, these gases were removed from both portions of the sample. A column of sodium hydroxide deposited on as-

[1] The use of the katharometer for estimations of ethyl iodide was first suggested in this laboratory by Dr. L. E. Bayliss.

[2] Made by Leeds and Northrup, Philadelphia. In the form used the platinum cell wires were covered with a thin layer of glass.

[3] When a sample containing ethyl iodide was passed through a tube immersed in a bath of alcohol and solid carbon dioxide, an amount of ethyl iodide exerting a pressure of approximately 0.14 mm. Hg escaped condensation. Presumably this represents the vapor pressure at this temperature (−72°C., Smithsonian Physical Tables).

bestos ("ascarite," Stetser, 1924), of magnesium perchlorate anhydride ("anhydrone," Willard and Smith, 1922) and of phosphorus pentoxide proved satisfactory for this purpose. With such an absorbing column mix-

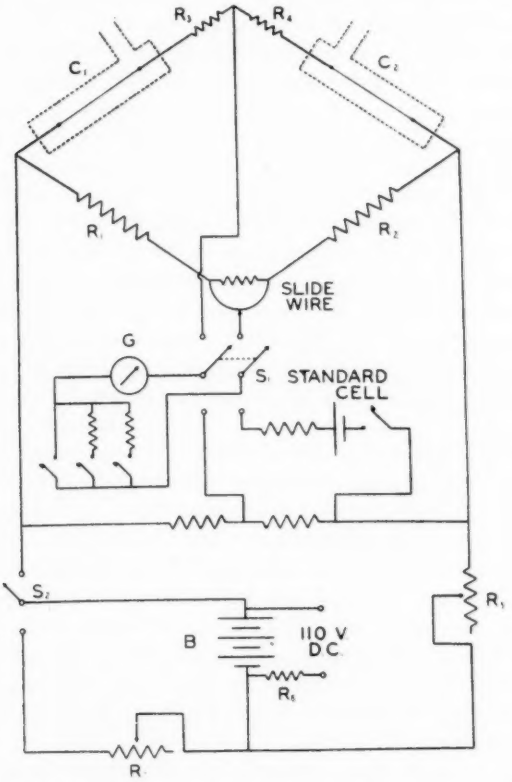

Fig. 1. Diagram of electrical connections for the katharometer. B, storage battery; G, sensitive galvanometer; C_1, C_2, katharometer cells; R_1, R_2, ratio arms; R_3, R_4, compensating resistances; R_5, resistance to adjust potential applied to bridge; R_6, resistance to permit continuous charging of battery; R_7, resistance to replace cell wires when they are disconnected; S_1, switch controlling position of galvanometer in circuit; S_2, switch to disconnect cells from battery.

tures containing as much as 32 per cent of carbon dioxide showed the same *zero point*[4] as that secured with room air.

[4] The term "zero point" will be used to denote the position of the slide wire which gives no deflection of the galvanometer after the apparatus has been filled with room air or any gas mixture the composition of which, after passing through the absorbers, undergoes no further change at the temperature of liquid air.

Compensation for the differences in the zero point resulting from the varying proportions of oxygen and nitrogen in respiratory samples and the unavoidable inequalities in the cells was secured by the insertion of an appropriate resistance at the end of one cell or the other (R_3 or R_4, figure 1).[5] After such an adjustment the apparatus was found to give the same zero point with 4 per cent oxygen in nitrogen as it did with room air, a difference far beyond the range encountered in samples of respired air.

As the quantity of alveolar air available for analysis was too small to permit satisfactory washing out of the cells, absorbers and connecting tubing, these parts of the apparatus were evacuated before filling them with the sample. A capillary tube was inserted at the inlet to prevent damage to the wires of the katharometer by a sudden inrushing of gas, and to decrease the rapidity of passage of the gas over the absorbents. To insure completeness of replacement, an initial evacuation and partial filling is customarily followed by a second evacuation and final filling before the slide wire is adjusted. To shorten the time required it is our custom to fill the cells to a pressure less than that of the atmosphere (approximately 45 cm. Hg), the pressure used being kept constant within 1 cm. in any one series.

As it seems possible that the cell wires or their terminal connections might be damaged by the heat generated during long continued evacuation, the circuit is disconnected before evacuation and is not reconnected until the pressure in the cells is greater than 10 cm. Hg. In order that the conditions of convection about the cells may be the same, each slide wire reading is made the same length of time after the reëstablishment of the circuit. The periods during which the circuit remains connected are uniform in length, as are also the periods during which it is disconnected.[6]

It has been found advantageous to use a synchronous clock motor to continue the regular connection and breaking of the circuit during periods when the apparatus is not in use.

Determination of the zero point. Before placing the flasks of liquid air in position at the beginning of a day, the spiral tubes and the cells must be filled with room air and evacuated at least twice. This removes the ethyl iodide condensed in previous experiments which was vaporized as the helical tubes approached room temperature. If this is done the zero point will remain constant after two or at the most three determinations following the installation of the liquid air flasks.

[5] Peters, quoted by Daynes (1933, p. 84) has used a similar resistance to correct for variations in current through the cells.

[6] To prevent change in the potential of the battery it is connected to a resistance (R_7, fig. 1) equivalent to the cells.

The steps involved in the determination of the zero point may be summarized as follows:

Time	
0	1. Disconnect cells from storage battery.
	2. Evacuate cells, desiccator and connecting tubing.
	3. Allow cells to fill through capillary from room air to a pressure of approximately 45 cm. of Hg.
	4. Repeat 2 and 3.
3 minutes	5. Connect cells to storage battery.
	6. Adjust potential applied to cells by comparing it with the potential of a standard cell.
5 minutes	7. Adjust slide wire until galvanometer shows no deflection and record slide wire position. Disconnect cells from storage battery.

The succeeding determination may be secured by an immediate repetition of the process.

A second reading of the zero point was customarily made at the end of any series of ethyl iodide determinations. Usually it agreed with the first. In the occasional cases where the two have failed to agree a zero point obtained by interpolation on the basis of time gave accurate results.

Estimations of relative amounts of ethyl iodide in known dilutions of its vapor. When determining the concentration of ethyl iodide in any sample the operator goes through the same steps as in the determination of the zero point except that, in step number 3, the cells are filled from the sampling tube. The difference between the zero point and the new slide wire reading, which is due to the presence of the ethyl iodide, will be referred to as the *differential reading.*

When the granular absorbents were used it was found that the differential readings secured with samples of ethyl iodide were dependent, to a certain extent, upon the preceding concentrations of this substance to which the granular material had been exposed. Thus the differential reading for the first filling of the cells was approximately 90 per cent of the readings obtained for the third and subsequent fillings. It was found, however, that determinations made on a series of mixtures yielded results directly proportional to the ethyl iodide concentrations present in the samples, when the cells were filled as described, provided the concentrations of ethyl iodide diminished throughout the series.

In the calculation of cardiac output a knowledge of the absolute concentrations of ethyl iodide is not required, only the proportions of the concentrations in the four samples need be known. This makes it possible to test the accuracy of the instrument by comparing the differential readings of a series of known dilutions made from a single sample. For such a test a sample of ethyl iodide in air was prepared in a twenty-liter bottle,

equipped with a stirrer and a mercury manometer. After the differential reading of a portion of its contents had been determined, the bottle was evacuated to a measured pressure and allowed to refill with room air to atmospheric pressure. A second reading was followed by a second dilution, and this was repeated for the desired series.

The results of two series of estimations are shown in table 2. The initial filling of the cells was made with an ethyl iodide concentration approximately twice that customarily used in the spirometer. As it follows a

TABLE 2

Estimations of known dilutions of an ethyl iodide sample

PRESSURE IN 20 LITER BOTTLE AFTER EVACUATION	ELECTRICAL READINGS IN DIVISIONS OF KATHAROMETER SLIDE WIRE*		
	Observed	Computed from second reading	Difference
mm. Hg			
	237.0‡		
307.0‡	114.0		
485.0	71.5	72.2	−0.7
349.0	34.0	33.1	+0.9
585.0	24.5	25.3	−0.8
Average			±0.80
Barometer 765.0 mm. Hg			
	190.0‡		
427.0‡	119.3		
424.0	67.7	66.6	+1.1
423.0	36.4	36.9	−0.5
422.0	20.0	20.4	−0.4
Average			±0.67
Barometer 763.0 mm. Hg			

* The electrical reading for 5 mgm. of ethyl iodide per liter, the usual inspired concentration, is approximately 100 slide wire divisions.

‡ These data not used in the computations.

filling with room air its differential reading was disproportionately low, and was therefore disregarded. The remaining readings were compared with the values calculated from the reading of the second sample and the known dilution ratios. A series of 41 successive estimations of known dilutions, made in the course of 18 months, demonstrated that the average error of a single differential reading is 0.79 per cent of the usual inspired concentration, or 0.0006 per cent of ethyl iodide, by volume. The maximum error of a single reading in this series was 2.4 per cent of the usual inspired concentration.

THE DETERMINATION OF CARDIAC OUTPUT WITH THE KATHAROMETER. In order that the method of determining ethyl iodide just described shall be applicable to actual determinations of cardiac output by the method of Starr and Gamble (1928) it is obvious that the estimations in the calibrating experiments and those in the cardiac output experiments should be made under exactly the same conditions. To accomplish this, an ethyl iodide mixture having approximately twice the concentration of the in-

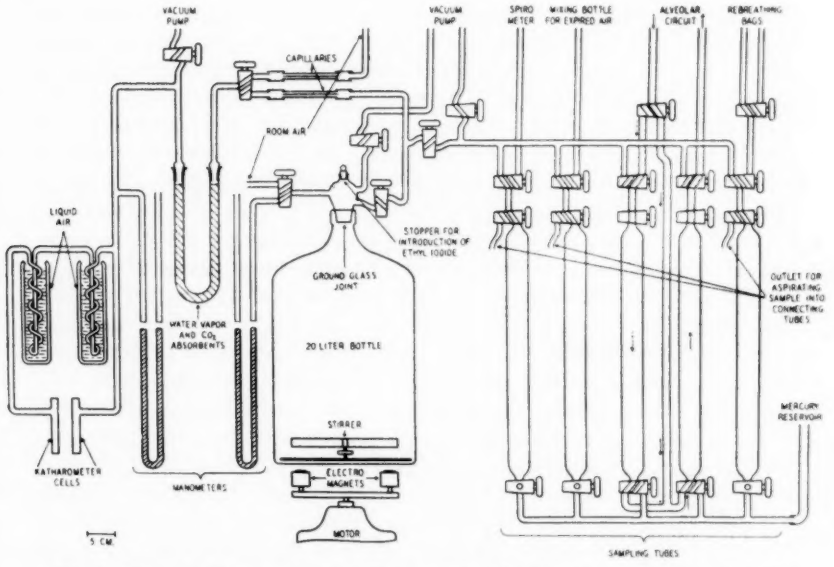

Fig. 2. Diagram of apparatus to collect samples of gas in determinations of cardiac output and to introduce them into the katharometer cells.

The following dimensions have been found satisfactory: Volume of each sampling tube, 300 cc. Internal diameter of drying tube, 1.0 cm. Length of drying columns: anhydrone 3 cm., ascarite 15 cm., anhydrone 3 cm., phosphorus pentoxide 10 cm., total 31 cm. Internal surface of glass spirals immersed in liquid air, 300 sq. cm.; corresponding volume, 19 cc. Length of each glass capillary, 10 cm.; internal diameter, 0.3 mm. Diameter of bore of alveolar circuit stopcocks, 4 mm.

spired air is prepared in the 20 liter bottle and used for the preliminary filling of the cells. The respiratory samples are then analyzed in the order inspired, expired, alveolar, and rebreathed air, an order which in all cardiac output determinations is one of decreasing concentrations. Since the conditions under which the control experiments were made are reproduced in the analyses of the respiratory samples, the accuracy of the two sets of observations may be expected to be the same.

The arrangement of the sampling tubes is shown in figure 2. Mercury was used as the displacing fluid. The tubing leading from the point of collection to the two tubes for alveolar air is washed out by the intermittent flow required for the collection of this sample. In the case of the three other samples the gas in the connecting tubes was replaced, just before collection, by a portion of the sample which was aspirated through the side opening of the 3-way cock at the upper end of the sampling tube.

Computation of results. In passing through the granular absorbents the respiratory samples are decreased in volume by absorption of CO_2 and water vapor and the concentration of ethyl iodide is thereby increased. As the final calculation is made from relative concentrations, and as all samples are saturated at room temperature, no correction need be made for the water vapor removed. The carbon dioxide, however, is not the same in each sample, and the differential reading secured must be multiplied by a factor which represents the ratio of the volume remaining after the removal of carbon dioxide to the total volume. That such a correction is justified over a wide range is shown by an experiment in which the differential reading of a sample of ethyl iodide in air was compared with readings secured after successive dilutions of the same sample with carbon dioxide. The reading of the undiluted sample was 104.5 divisions (5.2 mgm. per liter). Those with dilutions which gave concentrations of 14, 32, 40 and 51 per cent carbon dioxide were 104.0, 104.0, 103.0, and 103.5 divisions.

For the most accurate computations of the cardiac output the carbon dioxide content of each sample should be measured and the differential reading should be corrected accordingly. A great simplification in the calculation as well as in the analytical technique could be made, however, if it were possible to use a single factor to represent the combined effect of the three carbon dioxide corrections. To test the accuracy of such a procedure 35 cardiac output determinations were made in which the ethyl iodide concentration of each sample was corrected for the decrease in volume resulting from the absorption of the amounts of CO_2 found by analysis. The ratio of each of the cardiac outputs so computed to that calculated from the uncorrected differential readings was determined. The average value for the 35 ratios was 1.096. When this average factor was applied to each of the uncorrected values for cardiac output the average difference from the fully corrected value was found to be 1.65 per cent. Since these differences are not systematic and are much smaller than the unavoidable errors arising from other causes, the abbreviated form of calculation is justified.

The saving of time which this allows in the calculation may be seen by comparing the formulae for calculation by the two methods.

Fully corrected method:

$$\text{Cardiac Output} = \frac{(I - Z) - (E - Z)\frac{(100 - Exp)}{100}}{(A - Z)\frac{(100 - Alv)}{100} - (R - Z)\frac{(100 - Rebr)}{100}} \times \frac{Resp}{D.C. \times Temp.\ Corr.}$$

Abbreviated method:

$$\text{Cardiac Output} = \frac{(I - E)^7}{(A - R)} \times 1.096 \times \frac{Resp}{D.C. \times Temp.\ Corr.}$$

I = Slide wire setting for inspired air
E = Slide wire setting for expired air
A = Slide wire setting for alveolar air
R = Slide wire setting for rebreathed air
Z = Slide wire setting for room air = Zero point
Exp. = Carbon dioxide per cent in expired air
Alv. = Carbon dioxide per cent in alveolar air
Rebr. = Carbon dioxide per cent in rebreathed air
Resp. = Respiration in liters per minute
D. C. = Distribution coefficient for ethyl iodide (Cool, Gamble and Starr, 1934)

$$\text{Temp. Corr.} = \frac{(273 + \text{Room Temp.})}{(310)} \times \frac{(\text{Barom.} - 48.1)}{(\text{Barom.} - \text{Vapor Pressure at Room Temp.})}$$

In calculating cardiac output the values for the distribution coefficient of ethyl iodide were taken from the work of Cool, Gamble and Starr (1934). For the male subjects 6.6 was used, and for the females 6.4, corresponding to the normal erythrocyte counts of 5,000,000 and 4,500,000 per cu. mm.

The katharometer gives a marked saving of the operator's time in estimating cardiac output. A duplicate determination including the subject's breathing period, the collection of the eight samples, their analysis, and the calculation of the results requires 70 minutes or less. The analytical error is smaller than that of other methods, and its effect upon the calculated cardiac output is less than that of the other errors involved.

EXPERIMENTAL PROCEDURE. The subjects had taken no food or water for more than 12 hours and had lain quietly for at least 45 minutes before the first determination of cardiac output was made. In the lying position the subject rested on his back on a flat bed, with the head, but not the shoulders, resting on a thin pillow. For the determination in the sitting posture the subject sat in a straight chair. In standing, he leaned his hips against a firm vertical support, and was allowed to assume the position

[7] In the occasional determination where initial and final zero points do not agree these values for I, E, A, and R are corrected for the interpolated zero.

of greatest comfort and to change it moderately from time to time. For the determination in the inverted position, the subject was attached, by two long straps running over the shoulders and fastened near the feet, to a flat board which was then raised to the vertical position. Another strap running about the board at the level of the knees, and cords about the wrists, kept the limbs in place. After assuming a sitting, standing, or inverted position, the subject was connected to the spirometer within five minutes or less. Two determinations of cardiac output were made in each position. After the subject had assumed a vertical position an average interval of 18 minutes elapsed before the first determination, and of 30 minutes before the second. The period of about half an hour required for analysis of the final samples before the beginning of the next determination was spent by the subject lying comfortably on the bed. The order of the positions assumed was varied in the different subjects. When determinations were made in the lying, sitting and standing positions, the sitting position was either the first or the last. Individual determinations are given in table 3 for the inverted position. The remaining figures for the cardiac output are averages of the two duplicate determinations, except in the three cases in which one was unsuccessful owing to the loss of a sample, or to fainting or other abnormality of the patient.

Analysis of expired air taken at the time of collection of the ethyl iodide samples permitted the calculation of the oxygen consumption (Henderson and Haggard, 1925). As subjects breathing through valves often give an incorrect apparent respiratory quotient, the combustion of 1 liter of oxygen was assumed to produce 4.825 calories, corresponding to the average normal respiratory quotient of 0.82. The body surface was estimated according to the formula of Du Bois (9, p. 141), and comparison with the normal basal metabolism for the sex and age was made by means of Boothby and Sandiford's (1929) modification of the tables of Du Bois (9, p. 127).

Two or three observations of the pulse and respiration rates and of the blood pressure determined by auscultation over the brachial artery were made during each of the duplicate determinations, and the average of the results was used.

The subjects were normal medical students, physicians, and laboratory workers. The numbers by which they are indicated in table 3 are those used for the same subjects by Starr et al. (1934), in whose publication additional data regarding them may be found. The determinations there used are not, in all cases, the same as those given here. Subjects 43 and 48 are those indicated by S and G in earlier publications (25, 26, 27). The subjects will be identified by the same numbers in a forthcoming paper by Bazett, Cotton, Laplace and Scott on measurements of the velocity of the pulse wave.

Results. Results of consecutive determinations on 21 normal subjects

TABLE 3

The cardiac output determined by the method of Starr and Gamble in the lying, sitting, standing and inverted positions

SUBJECT	AGE	HEIGHT	WEIGHT	ORDER OF DETERMINATIONS	LYING			SITTING				STANDING			
					Pulse	O_2 consum.	Cardiac output l/min.	Pulse	O_2 consum.	Cardiac output l/min.	Cardiac output Sitting ÷ lying	Pulse	O_2 consum.	Cardiac output l/min	Cardiac output Standing ÷ lying
Males															
		inches	*lbs.*			*cc./min.*			*cc./min.*				*cc./min.*		
224	43	66.5	144	L. S.	52	161	4.4					82	224	3.7	0.84
220	48	68	144	L. S.	56	207	2.5					73	217	2.2	0.88
48	40	67.5	150	L. S.	61	252	4.0					83	284	3.6	0.90
48				S. L.	57	196	4.2					71	246	3.3	0.79
48				L. I. S.	59	252	3.9					78	300	3.3	0.85
225	33	70	115	S. L.	48	193	3.8					77	231	3.0	0.79
226	22	70	162	L. S.	72	242	3.6*					93	261	3.0	0.83
223	29	67	133	S. L.	64	196	4.3					78	223	4.0	0.93
228	37	68	140	L. S.	76	241	4.6*					84	262	3.5	0.76
43	38	72	183	S. L. Sit.	75	240	4.9	77	244	5.0	1.02	84	278	4.0	0.82
230	21	70	155	S. L.	66	253	4.2					94	238	2.6	0.62
231	23	68	175	S. L. Sit.	61	242	6.1	64	251	4.6	0.75	92	264	4.7	0.77
232	24	71.75	187	Sit. S. L.	65	258	5.4					100	286	4.4	0.82
103	28	68.5	140	L. I. S. Sit.	56	225	4.6	58	220	3.4	0.74	81	266	3.2	0.70
238	22	71.5	165	Sit. S. L.	59	240	5.3	68	243	3.8	0.72	77	286	4.3	0.81
Average (males)											0.81				0.81
Standard deviation											0.12				0.07
Females															
227	34	62	120	Sit. L.	72	189	3.3	91	198	3.2	0.97				
227				S. L. Sit.	65	176	3.4	87	201	2.9	0.85	102	209	2.9	0.85
152	27	66	133	S. L. Sit.	66	187	4.2	74	204	4.1	0.98	86	216	3.6	0.86
229	25	59	108	L. S.	92	171	4.2					119	211	3.2	0.76
233	46	62	115	S. L. Sit.	61	174	2.8	71	191	2.4	0.86	118	200	2.7	0.96
235	22	64	125	S. L. Sit.	79	163	2.2	80	153	2.1	0.95	93	179	2.0	0.91
51	32	71	150	S. L.	68	227	4.0					86	248	3.4	0.85
236	27	64	120	L. S. Sit.	62	187	3.9	70	196	3.3	0.85	81	202	3.5*	0.90
237	20	67	145	Sit. S. L.	70	207	5.0	88	216	4.5	0.90	96	197	2.9	0.58
Average (females)											0.91				0.83
Standard deviation											0.05				0.11
Average (all subjects)											0.87				0.82
Standard deviation											0.10				0.09

								INVERTED							
								First determination				Second determination			
48				L. I. S.	59	252	3.9	54	245	3.1*	0.81	55	298	3.6*	0.92
103				L. I. S. Sit.	56	225	4.6	82	257	3.4*	0.73	82	309	4.3*	0.93

* Values based on a single determination. Each of the other values for cardiac output is the average of two determinations.

are given in table 3. In each subject the cardiac output per minute is less in the standing than in the lying position. The average ratio of the standing to the lying output per minute is 0.82 with a probable error of the average of ± 0.013. In most cases the output in the sitting position was found to be intermediate between those in the other two, but with a distinct tendency to be nearer that found in the standing position. For the 10 subjects, the average of the ratios of the cardiac output per minute in the sitting to that in the lying position was 0.87 ± 0.02.

As previous observers have found a sex difference in the relationships of the cardiac output in the two positions, the results have been grouped in table 3 in two sections. The averages of the ratios in the two groups do not show a significant difference.

Owing to the well-known acceleration of pulse in the vertical position, the ratios of the outputs per beat are lower than those for the outputs per minute, being 0.76, with a probable error of ± 0.023 for the sitting/lying ratio and 0.60 ± 0.015 for the standing/lying ratio.

The arterio-venous oxygen difference (which may be calculated from table 3) is distinctly higher in each case in the standing than it is in the lying position, the average of the ratios of lying to standing being 1.41 ± 0.022. The a-v difference in the sitting position is intermediate between the two, the ratio to that in the lying position being 1.21 ± 0.03.

In the inverted position engorgement of the face and lachrymation made the subjects uncomfortable throughout. Tingling of all extremities occurred within 5 minutes and increased until in each second determination it resulted in unavoidable motions of the limbs. The dyspnea following the 30 seconds' rebreathing, which in other positions is scarcely noticeable, was subjectively intense.

In the second determination on subject 103, discomfort from the pressure of the shoulders against the straps led to muscular movements designed to lessen this. These exertions probably account for the observed rise in output.

Each of the four determinations is significantly lower than the cardiac output of the same subject in the horizontal position. Since the change to be expected from the discomfort and the muscular movements is an increase in the blood flow, the conclusion seems justified that in the inverted position the effect of gravity decreased the cardiac output. As in the other two vertical positions the output per beat is decreased and the arterio-venous oxygen difference is increased.

In table 4 are recorded the averages of the changes found in the remaining respiratory and circulatory data which accompanied the changes in position.

Discussion. Although the conclusion that the cardiac output is lower in the erect position appears justified, it would seem worth while to review the possible sources of error.

The accuracy of the determinations of ethyl iodide in the inspired and expired air could scarcely be affected by the position of the subject. The estimation of the ethyl iodide content of the arterial blood from that in samples of automatically collected alveolar air has been justified by experiments performed with the subject in the lying (Starr and Gamble, 1928) and the standing position (Starr and Collins, 1931).[8]

As it was realized that the change in rate and volume of respiration accompanying the alterations in position would change the quantity of air taken into the alveolar circuit at each breath, a control experiment was

TABLE 4

Comparisons of measurements of respiratory and circulatory functions in four positions of the body

	LYING		SITTING		STANDING		INVERTED*	
	Average values for all subjects	Standard deviation	Per cent of lying	Standard deviation	Per cent of lying	Standard deviation	Per cent of lying	Standard deviation
Number of observations	24		10		23		2	
Cardiac output, liters per sq. m. body surface	2.36	0.34	87	10	82	9	77	4
Output per beat, cc. per sq. m. body surface	36.8	8.0	76	11	60	10	69	19
A-V O_2 difference, cc. per liter	53.2	11.1	121	14	141	15	135	21
Pulse	65	9	114	10	137	18	118	26
Respirations per min.	12.4	3.1	105	19	106	16	149	29
Respiration, liters per sq. m. per minute	2.70	0.33	117	8	135	15	145	25
Systolic blood pressure, mm. Hg	103	7	105	6	106	9	105	5
Diastolic blood pressure, mm. Hg	82	10	108	8	110	9	102	1
Alveolar CO_2, per cent	5.56	0.32	94	3	87	3	88	3
Metabolism, per cent of normal basal (3)	91	8	104	6	114	9	106	9

* Initial determinations, only.

performed to test the effect of such changes. With one subject the negative pressure in the spirometer was varied so that in successive determinations the collection of the alveolar samples was made at the rate of 34, 38, 12, 18, 14 and 23 cc. per breath. The calculated cardiac outputs were 4.1,

[8] As the concentration of ethyl iodide in the alveolar air increases with extreme slowness, this comparison does not require an exact estimate of the time required for the blood to pass from the lungs to the artery where it is collected, thus making such a comparison less subject to error than those made when the subject is rebreathing from a bag in which the concentration of the foreign gas is rapidly changing.

4.6, 3.9, 4.2, 3.9 and 4.0 liters per minute. With another subject the flow in the alveolar circuit was varied without changing the negative pressure in the spirometer. Collections at the rate of 7 and 25 cc. per breath showed outputs of 4.4 and 4.6 liters per minute. In another experiment the corresponding values were 9 and 34 cc. and 3.8 and 3.4 liters. This indicates that relatively large changes in the rate of alveolar collection are without significant effect upon the calculated cardiac output.

The relatively slow change in concentration of ethyl iodide found to occur in the bronze bag during the course of rebreathing from it makes it appear improbable that an error would be caused by the change in rate of blood flow or in respiratory conditions resulting from the change of position. In the experiments here reported it would require an average increase of 16 per cent in the observed concentration of ethyl iodide in the rebreathed samples secured in the standing position to make the cardiac outputs in the two positions appear identical.

Although the work of Cool, Gamble and Starr (1934) indicated that the use of the average values for normal men and women of the distribution coefficient of ethyl iodide may result in an average error of about 4 per cent, such an error would have an equal effect on all determinations on the same subject and would not distort the ratios of the blood flows determined in different positions. Though Starr and Collins (1931) found no difference between the distribution coefficients determined with two samples of blood collected from the same subject in different positions, the changes in hematocrit reading found by Thompson, Thompson and Dailey (1928) after prolonged standing make it probable that there is a minor change in the distribution coefficient in the various postures. Calculations from their results and the distribution coefficient of plasma, 4.9 (Starr and Gamble, 1927) make it appear improbable that this could introduce an error of more than 4 per cent. This error, moreover, would make the bloodflow as here calculated unduly high in the erect positions, and would consequently strengthen the conclusion that the cardiac output is significantly greater when the subject is horizontal.

An attempt to evaluate the accuracy of any method of cardiac output is difficult because of the impossibility of absolute calibration. The test of reproducibility, so often put forward in defense of a method, gives no indication of the reliability of either the relative or the absolute values. It shows only the magnitude of the accidental errors. In the 53 duplicate determinations of these experiments each one differs from the other of the pair by an average of 6.6 per cent of their mean. This is approximately the degree to be expected from the average analytical error found in the calibration of the apparatus. From this, as well as from the probable error of the average ratios found in table 3, it seems fair to conclude that the apparent effect of posture on the cardiac output is not the result of analytical or of accidental errors.

Schellong and Heinemeier (1933) suggest that the disagreement between the uniformity of output in the lying and standing position which they observed, and the findings of the type here given may be due to the fact that all of their determinations were made between 4 and 8 minutes after the new position was assumed, and before the completion of circulatory adjustments to the accumulation of fluid in the legs or other changes. Our results, however, give no evidence of a continuation of such an adjustment between the time of the initial determination which was on the average 18 minutes after beginning to stand and the second which was made 12 minutes later. The average of all the second determinations, indeed, exceeds that of the initial ones by 3 per cent. Similar results were secured by H.-E. Bock (1934). He gives seven experiments in which successive determinations were made in the standing position. In four of these the second result was greater than the first, the average change for the entire series being an increase of 3 per cent.

SUMMARY

The katharometer has been adapted to the measurements of ethyl iodide in air required by the procedure of Starr and Gamble for determining the cardiac output in man. The average error of a determination of the relative concentration of ethyl iodide in the range in question is ± 0.04 mgm. per liter or ± 0.0006 per cent by volume.

The instrument permits a marked saving in the operator's time required. A duplicate determination of cardiac output including the analyses and calculation of results may be made in 70 minutes.

Cardiac outputs in the lying, sitting, standing and inverted positions have been compared in fasting subjects at rest.

The average value in the sitting position was 87 and in the standing position 82 per cent of that when lying. The corresponding values for the output per beat were 76 and 60 per cent.

In all cases the arterio-venous oxygen difference was greater in the erect than in the horizontal position.

In the vertical position with the head down, the cardiac output per minute and per beat was less, and the arterio-venous oxygen difference greater than when the subject was horizontal.

Comparisons are given of other circulatory and respiratory values in the various positions.

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THE MEASUREMENT OF THE OXYGEN CONSUMPTION OF IMMATURE RATS

J. E. DAVIS AND A. BAIRD HASTINGS

From the Lasker Foundation for Medical Research and the Department of Medicine, the University of Chicago

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The oxygen consumption of a colony of immature rats was measured monthly in order to determine the effect of increasing age on metabolism up to four months. The 4-month period was chosen not because rat immaturity ends exactly then, but because the oxygen consumption rate, which is changing rapidly and showing great variability during the maturing process, reaches a state where the variability is almost negligible at that time. The rats were kept in a room of fairly constant temperature, ranging from about 25° in winter to 28° in summer. They lived on a balanced weekly diet and fasted 16 to 21 hours before a determination. The rat colony numbered 136, consisting of 42 white males, 32 hooded males, 33 white females, and 29 hooded females. All of them were used for the first and second month's determinations, 127 for the third, 89 for the fourth, and 48 for those between the second and third month. That the same rats, only fewer, were used for the determinations subsequent to the first two did not appear to be of great importance; for it was found that the oxygen consumption differed but little from rat to rat of the same age, except around the third month.

The method of determining the oxygen consumption was a modification of that already described (1). This modification consisted in changing the burette oxygen measuring device for a Krogh type of spirometer, illustrated in figure 1. The scale G (see legend, fig. 1) may be read with an accuracy of half a division. This corresponds to 0.5 cc., or an error of 2 to 3 per cent in the case of rats. Alcohol checks carried out as previously described (2) resulted in oxygen consumption measurements deviating 0.1 to 1.0 per cent from the theoretical amounts. The characteristic feature of this spirometer is a self-compensating counterweight, which is described in the legend under figure 1.

Table 1 shows a typical record of successive five-minute oxygen consumption measurements obtained on a rat by such a spirometer and scale. It may be seen that if the operator makes careful and constant observations of the rat between readings, he can not only eliminate those readings

which show the effect of activity, but can also distinguish between the other readings as they are affected by the state of the rat. Three states of quietness may be recognized: first, one where the rat is wide awake; second, one where the rat seems to be sound asleep; and third, one where the rat is in an intermediate state between waking and sleeping. The first state is easily recognized, but has not been used because it is not considered as basal as the other two. The second state, designated for brevity as the sleeping state, is also easy to recognize; for in it the rat has all the characteristics of being sound asleep, and gives the lowest rate observed. This

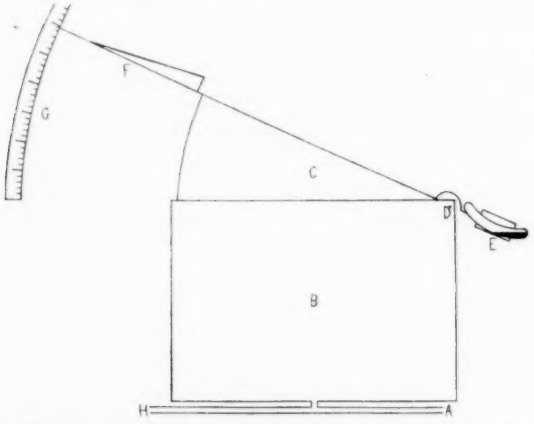

Fig. 1. Diagrammatic longitudinal section of spirometer. *A* indicates oxygen inlet; *B*, water chamber; *C*, gas chamber; *D*, fulcrum; *E*, counterweight; *F*, pointer; *G*, scale; and *H*, oxygen outlet. *E* is a counterweight consisting of a metal block carrying a curved tube filled with mercury and oil. Coarse adjustment is attained by moving the counterweight on its support. Compensation for the buoyant effect of the water as the spirometer descends is made by the flow of the mercury toward the fulcrum, *D*. The curved tube holding the mercury may be turned on its supports to provide the proper flow of mercury with respect to the fulcrum. The purpose of the oil is to steady the mercury movement. A manometer included in the system will indicate when the counterweight is properly adjusted.

state is usually preceded by a state, intermediate between the sleeping and wide awake, which is the hardest to recognize and gives a rate 15 to 20 per cent higher than the sleeping state. This intermediate state we have called the dozing state, and in it the rat opens and closes its eyes for brief intervals at a time, and seems on the verge of sleeping. A little experience enables one to distinguish between the sleeping and dozing states. Of the oxygen consumption rates about to be reported, some were found for the dozing state only, and others for the sleeping state only; but in most cases both rates were found as in the case of the rat of table 1.

TABLE 1

*Oxygen consumption record of 4 months old rat**

TIME	STATE OF RAT	O_2 CONSUMPTION		
		Per 5 minutes	Per period	Per kilo per 24 hours
minutes		*cc.*	*cc.*	*liters*
0	Active			
5	Active	41.0		
10	Active	38.0		
15	Quiet—awake	35.0		
20	Quiet—awake	32.5		
25	Quiet—awake	29.0	58 per 10 minutes	28.4
30	Quiet—awake	29.0		
35	Quiet—awake	27.0		
40	Dozing	25.0		
45	Dozing	24.5	99 per 20 minutes	24.3
50	Dozing	25.0		
55	Dozing	24.5		
60	Dozing	23.0		
65	Dozing	22.0		
70	Sleeping	21.5		
75	Sleeping	21.0	64 per 15 minutes	20.9
80	Sleeping	21.5		
85	Waking	23.0		

* Weight, 253 grams; temperature, 28.0°; barometric pressure corrected, 722.4 mm.; fasting period, 18 hours.

TABLE 2

Mean oxygen consumption of 136 immature rats

(Liters per kgm. per 24 hours)

GROUP	TWO MONTHS OLD				BETWEEN TWO AND THREE MONTHS OLD				THREE MONTHS OLD				FOUR MONTHS OLD			
	Number	Dozing	Number	Sleeping	Number	Dozing	Number	Sleeping	Number	Dozing	Number	Sleeping	Number	Dozing	Number	Sleeping
White males	29	38.8	32	32.1	11	36.8	8	30.6	11	31.7	28	27.0	25	25.5	6	21.2
Hooded males	19	39.5	26	32.1	7	37.4	8	30.5	20	31.4	23	26.5	9	25.6	2	21.7
White females	24	38.3	28	32.1	10	35.9	11	30.0	20	30.3	23	26.1	23	25.4	9	21.0
Hooded females	18	38.7	20	31.4	10	35.8	9	29.2	21	30.9	19	25.9	10	26.2	4	21.3
Mean of all	90	38.8	106	32.0	38	36.4	36	30.0	72	31.1	93	26.4	67	25.6	21	21.2
Mean—probable error		0.08		0.07		0.20		0.18		0.13		0.11		0.09		0.06
Mean—standard deviation		1.18		1.08		1.78		1.63		1.62		1.60		1.07		0.45
Mean—calories per sq.m. per 24 hours		1,007		832		1,001		825		894		759		779		645

The mean oxygen consumption values so obtained, except those for the first month, are shown in table 2. The first month's mean value, 39.5, for the dozing state, was not included in the table because it was obtained under conditions that were not comparable with those of the subsequent months. The one-month old rats had just been weaned, whereas the others had been on the standard diet for at least a month. It is probable that the 16 to 21 hours of fasting before the determination lowered the metabolism of the youngest rats the most (3). It will be observed that the males and females, both white and hooded, differed but little in their mean oxygen consumption values, probably because of experimental error and neglect of the effect of oestrus on the females. Therefore, both males and females have been included in one total group whose mean values are given on line 5. The values on this line, expressed in terms of liters per kilogram of body weight per 24 hours, as well as the same values on the last line, expressed in terms of calories per square meter per 24 hours (assuming the consumption of 1 liter of oxygen produces 4.7 calories), both show a decreasing oxygen consumption with advancing age. For all cases the probable error is so small as to indicate that the error due to inadequate sampling may be considered negligible. There is, however, the experimental error referred to; and, in addition, the error caused by imperfect judgment as to the state of the rat. In all cases the smaller standard deviation for the sleeping state indicates a small amount of deviation from the mean, which was probably due not only to a less variable condition of the rat but also to more accurate judgment regarding the sleeping than the dozing state. The greater standard deviations for both the dozing and sleeping states of the third, and between the second and third, months indicate a greater amount of deviation from the mean during these periods, which was probably due more to variability in the rat than in the operator's judgment as to its state. This variability, so noticeable about the third month, seemed to be well over by the fourth month. It may be added that other data in hand show that the oxygen consumption rate of the fourth month continues with little change through several succeeding months.

Benedict and MacLeod (4) found that "groups of young rats under two months of age have a higher metabolism in general than individual rats of from 4 to 12 months of age." Mitchell and Carman (7) have also reported that the metabolism of rats 30 to 40 days old is higher than that of rats 90 to 190 days of age. Benedict and MacLeod (4), Green and Luce (5), Lewis and Luck (6), Mitchell and Carman (7), and many others have reported the heat production of rats that are fairly comparable with the four-month old rats of table 2, whose mean sleeping rate was 645, and mean dozing rate 779, calories per square meter of body surface per 24 hours. It is noteworthy that the values reported by these authors all lie between 644 and 800, except two which lie at either ex-

tremity. It seems very probable, therefore, that these values represent a few of the most common combinations of sleeping, dozing and waking that are likely to occur during a determination.

It is suggested that the state of quietness of the rat, as well as its age and sex, should be carefully taken into account in metabolism work on this animal.

From the foregoing considerations the following conclusions may be drawn:

1. The oxygen consumption of 136 normal rats decreased with age up to the fourth month.

2. No difference was found in the oxygen consumption of the males and females of this series, up to four months of age.

3. The direct measurement of the oxygen consumption of rats and its correlation with their state permit one to distinguish between two quiet states, which have been designated as dozing and sleeping.

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THE CONTINUOUS MEASUREMENT OF THE VELOCITY OF VENOUS BLOOD FLOW IN THE ARM DURING EXERCISE AND CHANGE OF POSTURE[1]

S. H. PROGER AND L. DEXTER

From the Boston Dispensary, Boston, Mass.

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Knowledge of the changes produced in blood flow through the extremities of man by exercise and changes in posture remains incomplete despite the fact that considerable theoretical as well as practical importance attaches to the subject. The fragmentary and conflicting character of the evidence is due not to a lack of interest on the part of investigators but to the technical difficulties in the continuous measurement of blood flow in man.

Gibbs (1) has recently described a thermo-electric blood flow recorder in the form of a needle. This instrument actually measures velocity flow only. Experience has shown that if the needle is thrust into a vessel a clot will rapidly form around it and if the instrument is not moved it will reach a steady state in which it will record changes in velocity flow for a period of hours. The evidence obtained with the instrument cannot ordinarily be interpreted volumetrically, but with a vessel of fixed calibre the method can be used to measure volume flow as well as velocity flow since under such a condition volume flow is directly proportional to velocity. Also when the velocity is unchanged or increased with an increase in the calibre of the vessel, qualitative changes (increase) in volume flow can be assumed as is also the case with unchanged or decreased velocity (diminished volume flow) when the calibre of the vessel is diminished. Gibbs' method requires nothing more than a venipuncture and is therefore adapted for use on human subjects. Where qualitative evidence of changes in blood flow is significant, the needle flow recorder has obvious advantages.

Gibbs uses a hollow needle into which he inserts the flow recorder which he has made up as a stilet. The length of this stilet is so adjusted that the tip with the heater and thermojunctions contained in it projects just beyond the needle into the blood stream. We have had the junction built into a needle, the heating circuit and hot junction lying along the bevel of the needle tip, the cold junction being situated 3 to 4 mm. behind the hot junction and adjacent to an opening in the shaft of the needle. This

[1] This study was made possible by a grant from the Bingham Associates Fund.

solid needle is less sensitive than the open needle but in our experience is far more durable and considerably easier to handle; also the process of insertion of the solid needle into the blood stream does not require the loss of any blood. The open needle has an advantage, namely, in determining by the withdrawal of blood whether or not the needle is in the vein, where this vessel is not easily visible. Curves obtained with the open and solid needles demonstrated no qualitative dissimilarities.

Testing of the apparatus. That the apparatus actually records the velocity of flow of a liquid is easily demonstrated by inserting the needle through a piece of rubber tubing through which passes a stream of water. An increase in the speed of flow produces a deflection which when recorded shows a rise of the curve, and a decrease in its speed a fall. In this manner one can demonstrate that the apparatus is sufficiently responsive easily to detect changes in flow of the magnitude which we were studying.

There is some danger that an instrument of this type would respond to changes in blood temperature which would be misinterpreted as changes in flow. In order to investigate this possibility the needle was immersed in a water bath and precautions taken to prevent convection currents. The temperature of the water was raised from 26° to 36°C. over a period of ten minutes, this change being greater than, and as quick as that occurring in exercise of the type we were studying (2). Fluctuations in the curves were negligible and could not have influenced the character of the velocity curves.

Compression of a vein just proximal to where the needle was inserted gave the following information: 1. If the apparatus were not in working order, no deflection of the galvanometer beam occurred. 2. A slight and delayed deflection to the side indicating a decreased flow showed that the needle was in perivascular tissue. 3. An immediate and wide deflection to the side indicating a decreased flow showed that the needle was in the blood stream.

METHOD. The following observations were made on normal subjects who were laboratory workers or patients without cardiovascular lesions. Two different veins were used in these experiments: 1, any superficial vein of the forearm, and 2, the median basilic vein. Since the latter drains for the most part the deep structures of the forearm, receiving only one small superficial branch, we assumed that the results obtained in using this vein were indicative of velocity changes occurring in the deep vessels of the forearm. The subject remained relaxed and quiet until the velocity curve came to a resting level before the actual experiment began. Basal conditions were considered unnecessary.

RESULTS. *Velocity relationship in deep and superficial veins of the forearm in exercise.* Velocity curves were obtained with the needle *a*, in the median basilic vein, and *b*, in a superficial vein of the forearm. Exercising

the muscles of the hand and forearm by opening and closing the fist consistently produced in the median basilic vein a considerable and rapid increase of the blood velocity as was to be expected. There was a quick decrease in velocity of blood flow as the exercise was discontinued. This result agrees with that of Hewlett and Van Zwaluwenburg (3) who demonstrated an increase in the velocity flow of the forearm during similar exercise some three to eight times over the flow in the arm at rest by recording the rate of swelling of the arm when the venous return was blocked.

The velocity of flow in the superficial vein, however, responds to hand exercise by a slight but definite slowing, although no gross change in the calibre of the vessel can be detected. This is apparently not due to the environmental temperature because this temperature remains unchanged during the experiment and liberation of heat produced by muscular contraction would manifest itself by vasodilatation or by increased velocity of the blood in the vein under consideration. It appears more likely that there is in exercise an antagonistic response between the superficial and deep veins of the arm. Such a response may indicate an actual shunting of blood from the surface to the deep structures where the demand for blood rises as a result of increased metabolism on the part of the muscles.

Response of blood velocity to general exercise. L. B. Ellis (4) in studying the circulation time with sodium cyanide of subjects whose exercise entailed chiefly the lower extremity (pedalling on a stationary bicycle), suggested that the velocity of venous flow in the inactive arms might remain unchanged or be less than that at rest. However, he concluded that the blood velocity in the arm was actually increased because the venous blood had a higher degree of oxygen saturation during this type of exercise than at rest.

Velocity curves were obtained on subjects sitting quietly on a stationary bicycle with the needle inserted in the median basilic vein. After the velocity curves became level, the subject pedalled the bicycle vigorously for several minutes at a rate of 92 times per minute and with loads varying in the different experiments between three and eight pounds. Despite evident dyspnea and a marked increase in pulse rate and arterial blood pressure, there was essentially no alteration in the velocity curve. Small irregular fluctuations were observed which are attributable to slight movements of the arm which, as was repeatedly noted during other experiments, were capable of causing changes of this character and magnitude. Although the legs were performing a notable amount of work and the circulation was responding with an increase in heart rate, the rate of flow of the blood in the arm was practically unchanged. Such results were regularly obtained on several normal subjects. From this fact, we conclude that in response to exercise the velocity of blood flow does not change equally in all parts of the circulatory system.

Changes in velocity of blood flow as measured by injection methods must therefore be interpreted as average changes over various portions of the vascular system and not as uniform changes, for not only may there be quantitative differences in the response of the blood flow to exercise in various parts of the circulatory system but qualitative differences as well.

Effect of arm positions on blood velocity. With the needle located in any sizeable superficial vein of the forearm, velocity measurements were obtained on subjects sitting quietly with their arms resting horizontally on the arm of the chair. The effect of raising and lowering the arm was studied. Raising the arm produced a momentary and moderate increase in velocity (probably due to gravity) followed by a collapse of the veins and a marked decrease in velocity below the level which was obtained with the arm in the horizontal position. A decrease in velocity accompanied by collapse of the vessels points to a definite diminution of volume flow. Lowering the arm produced, after an initial drop of the curve, distention of the veins plus a rise of the curve above the level obtained with the arm in the horizontal position, that is, an indication of a decided increase in the volume flow. Essentially the same qualitative curves were obtained by repeating the experiment with the needle in the median basilic vein. It would appear, therefore, that the blood velocity in the veins of the arm is reduced not by the positional obstruction to venous return but by other forces which hinder the blood flow. Tigerstedt (5), reaching the same conclusions on purely theoretical grounds, pointed out that whereas the blood velocity and the peripheral resistance in an *inelastic U*-shaped vessel remained the same in the up-right as in the inverted position, in an *elastic U*-shaped vessel, the peripheral resistance and consequently the velocity of the blood altered with changes in the position of the vessel. In the upright position, the vessel would become distended in its dependent portions from the weight of the fluid which it contained, the peripheral resistance would thereby decrease with a consequent increase in the blood velocity, and as a result of these last two factors the volume flow would increase. In the inverted position, the *U*-shaped vessel would partially collapse, the peripheral resistance would therefore increase and the velocity flow under the given head of pressure would decrease, and as a result, the volume flow would decrease.

CONCLUSIONS

1. The method recently described by Gibbs has been found useful and practicable as a means for measuring continuously the relative velocity of blood flow and in certain instances qualitative changes in volume flow.

2. In cases in which it is desired to record changes in velocity in a vessel which is visible, a solid needle in which hot and cold thermo-junctions have been incorporated has certain advantages over an open needle into

which the thin wires containing the junctions are threaded after venipuncture.

3. The velocity of blood flow in the superficial and deep veins of the arm behave antagonistically with exercise of that arm, the flow in the deeper veins becoming more rapid and in the superficial veins slower than at rest.

4. With moderately severe exercise involving chiefly the lower extremities (pedalling on a stationary bicycle), there is no change in the velocity of blood flow in the veins of the upper extremities.

5. In the superficial and deep veins of the upper extremities, the velocity of blood flow is slower with the arm held in the erect position, and more rapid with the arm hanging down, than when the arm is in the horizontal position.

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THE INTERACTION OF CORTICAL AND LABYRINTHINE IMPULSES TO OCULAR MUSCLE MOVEMENTS

E. A. SPIEGEL AND LOUIS ARONSON

From the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University, School of Medicine, Philadelphia, Pa.

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In previous studies it was shown that the vestibular nuclei play an important rôle in the cortical control of the lateral eye movements. Bilateral injuries of the vestibular nuclei change the direction of the movements of the eyes that are elicited by electrical stimulation of the frontal lobe (Spiegel and Teschler, 1929), and of the occipital lobe (Spiegel, 1933). Whereas stimulation of these cortical areas usually produces conjugate lateral movements to the opposite side, such stimulation yields chiefly vertical deviation of the eyes, if the vestibular nuclei on both sides have been previously injured.

Continuing these experiments we observed that in cortical epilepsy bilateral lesions of the vestibular nuclei have similar effects upon clonic movements of the eye balls (Spiegel and Aronson). These observations can be explained on the assumption that the vestibular nuclei act as a sort of relay station for corticofugal impulses that subserve lateral eye movements. When these pathways are interrupted by lesions of the vestibular nuclei, then only corticofugal impulses for vertical movements can reach the eyes.

Unfortunately, the anatomy of the pathways that conduct cortical impulses to the eye muscle nuclei is incompletely known. The posterior longitudinal fasciculus, the main pathway for cortical as well as vestibular conduction to the eye muscles, contains two groups of systems, ascending and descending fibres. Some authors (Spitzer, 1899, 1924; Muskens, 1914, 1930) assume that the descending fibers of this bundle carry the corticofugal impulses for the innervation of eye movements. These descending fibers, originating in the nuclei of the posterior commissure, receive centrifugal impulses from the globus pallidus, partly through the posterior commissure. Circumscribed lesions of the globus pallidus, however, do not produce a paralysis of gaze, nor does severance of the posterior commissure (Taga, 1929, working with Spiegel) prevent the appearance of lateral ocular movements when the cortex is stimulated. One must conclude that the ascending tracts in the posterior longitudinal

bundle carry cortical impulses to the eye muscle nuclei. These fibers originate in the vestibular nuclei. Whether corticofugal fibers reach the vestibular nuclei directly or whether neurons are intercalated between the cortex and the vestibular nuclei, remains to be studied.

Objection might be raised that the changes in direction of the eye muscle movements following injuries to the vestibular nuclei are not consequential to the interruption of corticofugal pathways to the ocular nuclei, but are perhaps due to concomitant lesions in the adjacent reticular substance, or due to shock upon a neighboring gaze centre (Bárány, 1907; Lorente de Nó, 1933) in these regions. It seemed desirable, therefore, to alter the state of excitation of these vestibular nuclei without injuring them mechanically. Such a change of excitation could be performed from the periphery, e.g., by one-sided extirpation of the labyrinth. Thus we first studied how the loss of normal equilibrium of the vestibular nuclei of both sides, following the abolition of impulses from one labyrinth, changes the effects of stimulation of the cortical eye centres.

Method. The experiments were performed on nine cats. The cortical foci of the frontal and occipital lobes were exposed on either side under ether anesthesia, or under combined dial and ether anesthesia, and the effects of stimulation of these areas by faradic stimulation were noted. Then the labyrinth was extirpated on one side, following the method of de Kleyn, or it was paralyzed by the injection of 40 per cent formalin into the round window. After nystagmus toward the normal labyrinth had developed, cortical stimulation was repeated. Other methods that produce a disorder of equilibrium between the vestibular nuclei of both sides will be described later.

To record the eye movements, motion pictures were taken, or the following method was used. A light vertical lever was placed in front of a photokymograph so that the shadow of its lower arm was projected upon the horizontal slit of the kymograph. The upper arm had a rectangular bend close to its end and terminated in a small loop. This was fastened to the anesthetized cornea.

Results and comment. As long as the labyrinths and the subcortical centers and pathways are intact, stimulation of the cortical centers of ocular movements induces chiefly conjugate deviation of the eyes to the opposite side, followed by clonic jerks in the same direction (fig. 1, 1 a and b). If the equilibrium between the vestibular nuclei of the left and right side is disturbed by one-sided labyrinth-extirpation, so that there is spontaneous nystagmus toward the normal labyrinth, stimulation of the cortex on the side of the labyrinth-extirpation tends to move the eyeballs in the direction of the remaining labyrinth; it can easily be understood that an increase of the preëxisting spontaneous nystagmoid movements is the result of such cortical stimulation.

In stimulation of the cortex on the side of the normal labyrinth during the nystagmus that follows unilateral labyrinthectomy, the cortical and the labyrinthine impulses have a tendency to produce opposite results. The cortical impulses should normally bring forth eye movements toward the side of the extirpated labyrinth, while the vestibular impulses tend to produce nystagmus with its quick phase toward the normal labyrinth. During this cortical stimulation various initial effects can be observed (compare protocols on following pages): 1, a more or less marked tonic

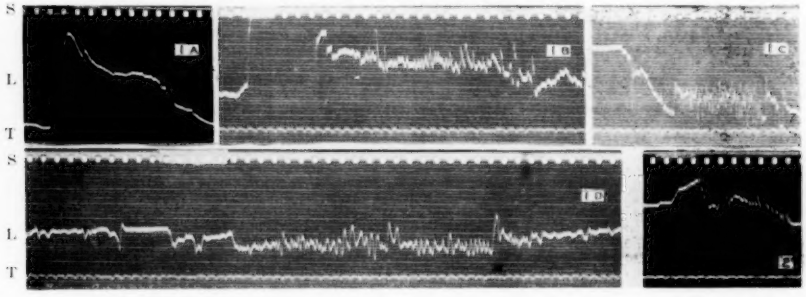

Fig. 1. Cat 1. Stimulation of the left occipital lobe, a and b, before; c and d, after extirpation of the right labyrinth. a shows mainly tonic, b, tonic-clonic eye movements to the right (upwards in the graph). c, tonic-clonic movements to the left. d, nystagmus to the left before and after the cortical stimulation, clonic eye jerks in the same direction immediately following the stimulation of the left occipital lobe. At *X* retraction of the eyeball.

Cat 2. Left labyrinth extirpated, nystagmus to right, disappeared in ether anesthesia. Stimulation of the right frontal lobe: conjugate deviation and clonic jerks of the eyes to right.

S: indicates the stimulation. L: is an optical record of the movements of the left eye, described in the text; upwards in the graph indicates eye movements to right. T: one second intervals. In order to utilize the full width of the recording paper, it was necessary to project the shadow of the lever in the beginning of 1a and 1b upon the lower half, in the beginning of 1c upon the upper half of the recording paper. This, however, did not influence the direction of the eye movements in the stimulation experiments.

deviation of the eyeballs towards either side (sometimes combined with a vertical component): 2, a quick tremor-like undulation of the eyeballs without a conspicuous quick phase in a certain direction; 3, a slight vertical movement of the eyeballs that continue their horizontal nystagmoid movements in the new position; 4, a diminution of the spontaneous nystagmus; 5 (rarely), there are dissociated movements of the eyeballs, e.g., one eye deviates horizontally to the opposite side, the other upwards or downwards, or a skew deviation of the eyes may be observed. These

various initial reactions are followed by a much more constant clonic response. Already during the cortical stimulation, or after its cessation, clonic jerks of both eyeballs toward the stimulated cerebral hemisphere develop, instead of the jerks toward the opposite cortex that normally follow the tonic phase (fig. 1, 1 c and d, 2). In other words, the clonic reaction shows a reversed direction compared with the normal response, and follows the direction of the preceding spontaneous nystagmus toward the remaining labyrinth. Sometimes the cortical stimulation results immediately in clonic jerks or tonic-clonic movements toward the stimulated hemisphere. The amplitude of these clonic reactions is much larger than that of the spontaneous nystagmus, their rhythm being usually the same as that of the clonic convulsions of skeletal muscles.

This reversed cortical reaction is illustrated by the following condensed protocol.

CAT. December 19, 1933. Left labyrinth extirpated, horizontal nystagmus to right.

December 20, 1933. Nystagmus to right persists.

11:30 a.m. Right cortex exposed under ether anesthesia, weak nystagmus to right

Time	*Stimulated area (Distance of coils)*	*Reaction of both eyes*	*Other reactions*
11:54	Right frontal lobe (4 cm.)	Slightly up, then increased nystagmus to right	
12:00	Right occipital lobe (6 cm.)	Tonic deviation to left; nystagmus to right	Pupil dilatation
12:01	Right frontal lobe (6 cm.)	Tremor (no definite direction to right or left), then increasing nystagmus to right	Pupil dilatation, general clonic convulsions
12:12	Right occipital lobe (6 cm.)	Tonic deviation to left, then nystagmus to right	
12:15	Right frontal lobe (6 cm.)	Upwards, then clonic jerks to right	General epileptiform convulsions
12:17	Right frontal lobe (6 cm.)	Deviation to left and downwards, then weak nystagmus to right	
4:45		Very weak spontaneous nystagmus to right	
4:53	Right frontal lobe (6 cm.) (ether anesthesia)	No distinct reaction during stimulation; it is followed by clonic jerks to right. Later slow nystagmus to right with small amplitude	
4:58	Right occipital lobe	Deviation upwards and to left, then strong nystagmus to right, outlasts the stimulation	
5:00	Right frontal lobe	Deviation upwards, then cloni to right	General convulsions

Time	*Stimulated area (Distance of coils)*	*Reaction of both eyes*	*Other reactions*
5:01 to 5:05		Very slight nystagmus to right	
5:06	Right frontal lobe	Upwards deviation, then cloni to right	
5:10 5:15 5:22	Right frontal lobe	During stimulation no distinct eye movements. It is followed by clonic jerks to right	Pupil dilatation
5:30		Very weak spontaneous nystagmus to right	

Thus in the competition between the antagonistic cortical and labyrinthine impulses, the latter eventually emerge the victor, and the clonic jerks of the eyeballs go in the same direction as the spontaneous nystagmus, toward the normal labyrinth, the reaction upon the cortical stimulation being reverse from the normal, namely, toward the stimulated hemisphere instead of toward the opposite cortex.

It can easily be shown that this reversal of the cortical response depends on the labyrinth that remains intact. If this is also extirpated on the same day, the abnormal cortical response that follows the first labyrinthectomy is no longer observed, and the stimulation of the cortical eye centers again elicits jerks of the eyeballs to the opposite hemisphere.

It seemed interesting to study stimulation of the cortical eye centers several days after unilateral labyrinthectomy, when the spontaneous nystagmus had already subsided. In this state of compensation of the lost labyrinth one may observe that the cortical impulse acts undisturbed upon the eye muscles, producing tonic-clonic movements to the opposite side, as under normal conditions. It may, however, also happen that the cortical stimulation elicits eye jerks in the direction of the previous nystagmus. In the latter case, the loss of the labyrinth is apparently not yet fully compensated for, and a latent tendency to spontaneous nystagmus still exists, which tendency can manifest itself after cortical stimulation. In the experiment reported below, for instance, stimulation of the occipital lobe alone produced eye jerks to the opposite hemisphere, while the reaction elicited by stimulation of the frontal lobe showed the tendency of the latent nystagmus to reappear.

The interaction of the cortex with vestibular impulses that originate not in the end organ but in the central nervous system itself can be studied, if the cortical response of the eyeballs is pitted against Bechterew's nystagmus. If, after unilateral labyrinthectomy or severance of the eighth nerve, one waits until the nystagmus subsides, and then extirpates the remaining labyrinth, a nystagmus toward the side of the first extirpation appears as if this labyrinth were still present (Bechterew, 1883). This nystagmus develops even when the forebrain is extirpated (Magnus and de Kleyn,

1923), and even after the diencephalon is removed, the roof of the midbrain destroyed, and the cerebellum excised (Spiegel and Démétriades, 1925). The authors last mentioned showed furthermore that the vestibular nuclei on the side of the second labyrinth-extirpation may be destroyed without preventing this nystagmus, but that destruction of the vestibular nuclei on the side of the first labyrinth-extirpation abolishes this nystagmus. They assumed, therefore, that the mechanisms which compensate for the loss of the first labyrinth are located in the vestibular nuclei on the side of this extirpation. In these nuclei a state of increased excitation develops after the first labyrinthectomy, due to their isolation from the end-organ and to impulses from higher parts of the central nervous system. This state of increased excitation balances the impulses which the central nervous system receives from the normal labyrinth. If these impulses are abolished, by extirpation of the remaining labyrinth, the vestibular nuclei on the side of the first extirpation, which are in a state of hyper-excitation, alone send impulses to the eye muscles, without the antagonistic impulses from the opposite side, and Bechterew's nystagmus towards the side of the first extirpation results. The following experiment illustrates the effect of cortical stimulation upon the eyeballs during Bechterew's nystagmus.

CAT. October 2, 1933. Left labyrinth extirpated, strong nystagmus to right develops that continues during the next days; the head is turned in such a way that the left ear is lower than the right.

CAT. October 7, 1933. No nystagmus, posture of the head unchanged.

October 9, 1933. Idem.

10:30–11:00 a.m. right cortex exposed,

11:03 slow spontaneous eye movements to left side (in one minute the right eye moves 7 times, the left eye 4 times)

Time	*Stimulated area (Distance of coils = 7 cm.)*	*Reaction of both eyes*	*Other reactions*
11:09	Right frontal lobe	Deviation to left; after the stimulation few jerks to right and upwards	
11:12	Right frontal lobe	Tonic-clonic upwards	General convulsions
11:14	Right occipital lobe	Tonic-clonic to left (overlast the stimulus)	
11:16	Right frontal lobe	Upwards during stimulation, then nystagmus downwards and to right	
11:19		Spontaneous up and downward movements	
11:21	Right frontal lobe	Upwards, then nystagmus up and to right	
11:30	Left cortex exposed		

Time	*Stimulated area (Distance of coils = 7 cm.)*	*Reaction of both eyes*	*Other reactions*
11:35	Left frontal lobe	Right eye up, left eye to right (tonic-clonic)	
11:42	Left occipital lobe	Right eye up, left eye to right; then cloni of both eyes to right	
11:46	Right occipital lobe	Tonic deviation to left	
11:47		Spontaneous nystagmus to right (both eyes); later slow up and down movements of the right eye (superficial ether anesthesia)	
11:49	Right motor cortex	Upwards (tonic), then clonic upwards and to right	
12:10		*Right labyrinth extirpated*, nystagmus to left	
12:25	Left motor cortex	Clonic jerks to left (much larger amplitude than the preceding nystagmus)	General convulsions
12:29	Left occipital lobe	Deviation to left, then nystagmoid movements downwards	
12:33		Weak spontaneous nystagmus to left	
12:35	Left motor cortex	Deviation downwards, then clonic jerks to left (overlast the stimulation) later: weak, slow spontaneous nystagmus to left	The eye jerks have the same rhythm as the general convulsions
12:36	Left occipital lobe	Deviation and then clonic jerks: right eye upwards and to right, left eye downwards and to right; later again weak nystagmus to left	
12:38	Left motor cortex	Deviation downwards and to right, then clonic jerks to left	
12:40	Right motor cortex	First spasmodic tremor, then strong cloni to left	The eye jerks have the same rhythm as the general convulsions
12:41	Right occipital lobe	Increases the weak spontaneous nystagmus to left	
12:43	Left motor cortex	Same reaction as at 12:40 (more marked on right eye than on left)	
12:44	Left occipital lobe	Skew deviation (left eye downwards, right eye upwards and somewhat to right); then cloni in the same direction	
12:45	Left motor cortex	Cloni to left	
12:46	Left occipital lobe	Deviation to left, then weak rotary nystagmus to right. After the stimulation: cloni of right eye upward and to left, of left eye downward and to left	
12:48	Left motor cortex	Cloni to left	General convulsions

Thus the stimulation of the cortex during Bechterew's nystagmus gives a result similar to that due to the interaction of cortical impulses and nystagmus after unilateral labyrinthectomy. Especially if clonic eye jerks develop, the labyrinthine impulses eventually determine the direction of the eye movements, even if the cortical impulses tend to produce an opposite effect.

These results seem at first sight contradictory to some observations of Bárány and C. and O. Vogt (1923) who studied on monkeys (Macacus) the influence of cortical stimulation upon caloric nystagmus. They produced for instance nystagmus toward the right side by cold water irrigation of the left ear. Stimulation of the eye center in the left frontal lobe did not change this nystagmus or accelerated it only, while stimulation of left sided occipital centers not only increased the frequency and lowered the amplitude of the nystagmus toward the right side, but even produced a deviation of the eyeballs to the left. Caloric nystagmus toward the side of cortical stimulation was inhibited or even reversed by such stimulation. The frontal lobe proved more effective in producing such a reversal than the occipital lobe. Thus Bárány and C. and O. Vogt observed a reversal of the direction of nystagmus by cortical stimulation, whereas in our experiments labyrinthine impulses proved able to reverse the effect of cortical stimulation upon the eye movements. Yet the results of these two groups of observations can be understood from a common point of view, if one considers the relative strength of the competitive cortical and labyrinthine stimuli. Calorization, particularly if continued for a while, is a weaker stimulus than the acute disturbance of equilibrium between the vestibular nuclei of both sides produced by unilateral labyrinthectomy. In previous experiments it was shown (Spiegel and Aronson, 1933) that continuous calorization of the normal labyrinth can lower the frequency of spontaneous nystagmus that follows extirpation of the opposite labyrinth, but that it is unable to suppress this spontaneous nystagmus. Thus the question arose whether stimulation of the cortical eye centers during calorization of one labyrinth produces an effect differing from that of cortical stimulation performed a few hours after unilateral labyrinthectomy. Continuous caloric stimulation of the labyrinth was performed by introducing in the external or in the middle ear the U-shaped apparatus that we described in a previous study of continuous stimulation of the labyrinth with sustained nystagmus (1933).

The following condensed protocol shows such an experiment.

CAT. October 20, 1933. Continuous stimulation of the left labyrinth with cold water (cannula in the cavum tympani, temperature of the outflowing water 22°C.) nystagmus to right.

Time	*Stimulated area (Distance of coils = 7 cm.)*	*Reaction of both eyes*	*Other reactions*
11:50	Right occipital lobe	Slight deviation to right, nystagmus to right continues	Cloni of left ear
12:08	Right frontal lobe	Convergence	General convulsions
12:10	Right frontal lobe	Deviation to left	General convulsions
12:13		Nystagmus to right	(Outflowing water 21, 5°C.)
12:14	Right occipital lobe	Deviation to right, nystagmus ceases; returns after stimulation	
12:15	Right frontal lobe	Amplitude of the cold water nystagmus to right becomes smaller	
12:17	Right occipital lobe	Tonic deviation to left, interrupts the cold water nystagmus	General convulsions
12:20	Right frontal lobe	Slight deviation to left, interrupts the cold water nystagmus	General convulsions after the stimulation
12:28	Right occipital lobe	Marked deviation to left, nystagmus ceases	
12:45		*Destruction of left labyrinth* (formalin injection into the round window)	
2:45		Nystagmus to right	
2:50	Right occipital lobe	Slight tonic deviation to left, in this position rotary nystagmus to right develops. Then general convulsions appear accompanied by vertical eye jerks. After the convulsions: nystagmus to right	
2:55	Right frontal lobe	Deviation to left, then cloni to right (large amplitude!)	General convulsions
2:58		Spontaneous nystagmus to right (small amplitude)	
3:00 and 3:07	Right frontal lobe	Reaction as at 2:55	
3:10 and 3:12	Right occipital lobe	Slight deviation to left followed by rotary cloni to right (large amplitude). In the intervals between the stimuli: spontaneous nystagmus to right (has much smaller amplitude than the cloni)	

The first part of this experiment shows, in agreement with the observations of Bárány and C. and O. Vogt, that the cortical stimulation can suppress or diminish the caloric nystagmus, which returns after the cortical stimulation ceases. If then the labyrinth that was previously douched with cold water is removed, and stimulation of the cortical eye centers on the side of the normal labyrinth is repeated during the ensuing spontaneous nystag-

mus, initial eye movements in various directions (mainly to the opposite hemisphere, see above) may be observed; yet even during the cortical stimulation, clonic jerks in the direction of the spontaneous nystagmus[1] may develop and may outlast the cortical stimulation. In other words, while the cortical impulse suppresses the caloric nystagmus, the interaction of the cortical stimulation and of the spontaneous nystagmus after labyrinthectomy results, even during the cortical stimulation, in eye jerks in the direction of the remaining labyrinth. In the first case the cortex, in the second case the labyrinth, proves to be stronger in competition. We have here to do with a mutual influence of centripetal impulses from the end organ and corticofugal impulses, the result depending on the relative strength of the interfering reactions. This seems to be in analogy to certain observations on skeletal muscles. In experiments of Graham Brown and Sherrington (1912) on monkeys, for instance, a flexor reflex could suppress or even reverse a cortical extensor reaction, while a stronger cortical extensor reaction could break through a weak flexor reflex and suppress it for a while.

It seems difficult to lay down rigid rules in regard to the different effect of frontal and of occipital stimulation upon the caloric nystagmus in cats. In one and the same experiment one could find, for instance, first, that the stimulation of the occipital lobe had a weaker effect, and later that it had a stronger effect than the frontal stimulation, upon the caloric nystagmus toward the stimulated hemisphere. Sometimes no certain difference between the two cortical areas in regard to their influence upon the nystagmus could be found. The fact that definite, constant, differences in the influence of the frontal and of the occipital lobe upon the caloric nystagmus were missed in the cat's brain does not exclude, of course, that such differences may exist in the more developed cortex of monkeys, as stated by Bárány and C. and O. Vogt.

It was shown that the direction of eyeball movements, in cortical stimulation, can be influenced not only by mechanical injuries to the vestibular nuclei (Spiegel and Teschler, 1929; Spiegel, 1933), but also by a change in their state of excitation due to labyrinthectomy. While mechanical lesions of the rhombencephalon could involve also the hypothetical gaze centers in the reticulate substance, this region remained intact in the present series of experiments. These experiments show how a spontaneous nystagmus can determine the direction of cortical eye reactions. In interpreting the results, it must be borne in mind that cortical impulses for horizontal eye movements, as already mentioned, have to pass through the posterior longitudinal fasciculus in order to reach the eye muscle nuclei. Even several weeks after severance of this bundle, cortical stimulation

[1] Occasionally eye jerks in the vertical direction were noted.

failed to elicit horizontal conjugate deviation of the eyeballs (Spiegel and Tokay, 1930). While pathways from the vestibular nuclei into the posterior longitudinal fasciculus were repeatedly shown (Van Gehuchten, 1904, Leidler, 1916), it is not known that cells of the reticulate substance send fibers into this bundle. In connection with these facts, our experiments seem to corroborate the conception that the vestibular nuclei are intercalated in the pathway of the corticofugal impulses for horizontal eyeball movements.

SUMMARY

1. The cortical centers of eye movements were stimulated after unilateral labyrinthectomy, during Bechterew's compensatory nystagmus, and during caloric stimulation of the labyrinth.

2. The interference of the spontaneous nystagmus after loss of one labyrinth, as well as of Bechterew's nystagmus, with cortical impulses which tend to move the eyeballs in the opposite direction, eventually results in clonic eye jerks in the direction of the nystagmus, towards the stimulated cerebral hemisphere instead of toward the resting hemisphere.

3. In caloric stimulation of the labyrinth, the vestibular reaction was weaker than the cortical, the corticofugal impulse suppressing a nystagmus toward the stimulated hemisphere.

4. It is assumed that the interference of the corticofugal with the labyrinthine impulses takes place within the vestibular nuclei.

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PERSISTENCE OF COCHLEAR ELECTRICAL DISTURBANCE ON AUDITORY STIMULATION IN THE PRESENCE OF COCHLEAR GANGLION DEGENERATION[1]

JOHN GUTTMAN AND S. E. BARRERA

From the Department of Neuropathology, New York State Psychiatric Institute and Hospital, New York

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The fact that sounds entering the cat's ear can be reproduced from either the 8th nerve or the bony cochlea of the same side by suitable electrical transformation, is well known and generally accepted. Concerning the anatomical elements participating in the transformation of sound into the electrical responses as recorded by the detecting apparatus, there is as yet considerable difference of opinion. This involves the question as to the parts played by nervous and non-nervous elements in the effect as obtained from either the 8th nerve or from the bony cochlea.

Wever and Bray (1), in their initial studies on this phenomenon, felt that the effects which they obtained from the 8th nerve contained a large neurogenic element and were essentially action currents originating in the the nerve itself. They discussed, however, the possibility of mechanical origin of the effect.

Adrian (2), in an initial criticism of this work, felt on the other hand, that the effects which Wever and Bray had obtained and which he obtained in addition from the bony cochlea, were due to some microphonic action of the cochlea itself, rather than to an action current in the nerve proper.

Later, Adrian, Bronk and Philipps (3) modified this opinion to some extent, when they concluded that it seemed probable that the potentials in the cochlea are due to nerve structures of some kind. Their results indicated to them that the Wever and Bray effect was due to living cells of some type. They stated, however, that it appeared probable that the nerve fibers were not the only structures giving rise to the large sized potential changes in the cochlea.

More recently, Adrian (4) restated the possibility that "the potential changes in the cochlea may be non-nervous or they may be due to the nerve endings or nerve cells and this may account for their great intensity."

[1] This research was assisted by a grant from the Committee on Scientific Research of the American Medical Association.

Davis, Saul, and their co-workers (5–12) in a large series of experiments on cats, concluded essentially that there are at least two distinct electrical phenomena involved in the hearing of a cat, namely, true action currents and "spread." The "spread," they felt, probably originates in the cochlea and spreads to all the tissues of the head. They felt that it is probably involved in the transformation of sound by the cochlea into nerve impulses. The auditory action current, on the other hand, is highly localized to the nervous pathways. In one animal with congenital deficiencies in one organ of Corti the authors reported absence of the cochlear response from the round window and from this case and others they concluded that the cochlear response seems to be "closely associated with mechanical vibrations in the inner ear, most probably the organ of Corti."

One of us (Guttman, 13) repeated the experiments of Wever and Bray on narcotized but non-decerebrated cats. In addition, he severed the 8th nerve and found persistence of the effect when led off from the cochlea. Davis and Saul recently confirmed this observation. Guttman suggested that the cochlear effect was independent of the nerve effect and that it might act as an electrical stimulus to the nerve.

In light of his findings concerning the persistence of the effect after section of the 8th nerve in his experiments, Guttman proposed to study the persistence of the cochlear effect in cats at various times after section of the 8th nerve, in the hope that section of the nerve might lead to subsequent degeneration of the intracochlear nervous elements. That such degeneration was possible was seen from the work of Wittmaack (14), who found that, after section of the 8th nerve in the cat, the cochlear ganglia, nerve fibers and hair nerve cells, disappeared. Such a degeneration would eliminate the nerve elements in the cochlea. Similar degeneration of the intracochlear nerve elements, associated with destruction of the 8th nerve intracranially, was reported by Crowe (15) in two human cases.

With the end in view of studying the cochlear effect after the disappearance of the intracochlear nerve elements, a series of cats was subjected to intracranial section of the 8th nerve. This was carefully performed, with severing of the artery in some cases, and avoidance of the artery in other cases. Various periods of time, up to longer than six weeks after the operation, were then allowed in order to permit the degeneration of any nerve structures effected by the lesion. Individual animals were then subjected at various intervals, after the operation, to exposure of the cochlea and stimulation by sound, with recording of the electrical effect as led off from the cochlea. The recording apparatus was a two electrode-3 stage-amplifying system, in the output circuit of which was a telephone receiver. The presence of the effect was determined by the reproduction in the telephone receiver of sounds applied to the ear, including a whistle and spoken or whispered voice. Finally the animals were killed and serial sections

were prepared of the cochlea and the brain stem in the region of the medulla to confirm the degeneration of the nervous elements in the cochlea and the section of the 8th nerve. H. and E. stains were used for most sections of the cochlea and the Weigert stain was used to study the degeneration of the nerve. In some more acute cases the Marchi stain was used. In all of the cases included in this report the section of the nerve was found to be complete, involving both the cochlear and vestibular portions.

The most important general physiological observation resulting from this series of experiments was that the cochlear response, as obtained from the bony cochlea, persisted unchanged, so far as our apparatus could

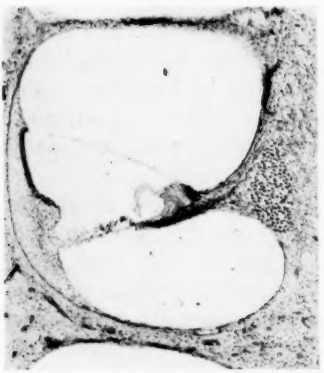

Fig. 1

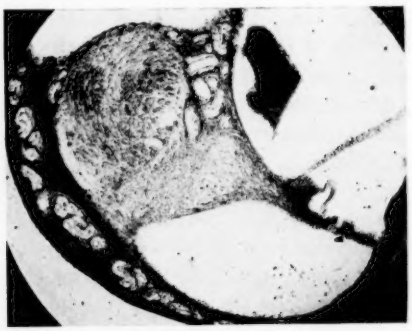

Fig. 2

Fig. 1. Photograph of a normal cat cochlea showing the clearly defined triangular shaped cochlear ganglion with its numerous round cells and connective tissue strands. H. and E. stain.

Fig. 2. Photograph of a similar ganglion as was seen in figure 1 but taken from a cat in which the 8th nerve of the same side had been cut six weeks before. The round cells are seen to have disappeared and all that remains is the connective tissue element. H. and E. stain.

detect, at all intervals tested, namely, from 10 days to 6 weeks after section of the 8th nerve.

Histologically the studies of the cochlea revealed that in the chronic stage following nerve section, confirming the observations of Wittmaack, the degeneration in the cochlear nerve had extended peripherally into the cochlea so as to produce disappearance of the cells of the cochlear ganglia. The exact status of the nerve endings of the organ of Corti still is under investigation. The degeneration occurred, as it did in the work of Wittmaack, even when the artery was uncut. Figure 1 shows a microphotograph of a section of the normal cat cochlea, stained with an H. and E. stain, showing the normal triangular shaped ganglia with its numerous round

cells and connective tissue. Figure 2 is a microphotograph of a section of a cat cochlea in which the 8th nerve had been cut on the same side six weeks previous to testing of the animal. The cochlear ganglion is seen to be completely degenerated, with disappearance of its cells and nerve fibers. In this animal, as in all other similar animals, the cochlear effect persisted. It is of interest to emphasize here that the effect persisted apparently regardless of the mechanism responsible for the disappearance of the ganglion because it persisted in all of our cases. This would indicate that even if, in some cases, the disappearance of the ganglion was due to interference with the blood supply, instead of being the result of uncomplicated nerve degeneration, the persistence of the effect in the absence of the ganglia is the important general observation.

SUMMARY

1. Section of the 8th nerve intracranially was performed in a series of cats.

2. After various survival periods following section of the 8th nerve, animals were studied to determine the presence of the cochlear response to sound stimulation. The active electrode was placed directly on the bony structure of the cochlea. The recording apparatus used was a two electrode-3 stage amplifying set-up.

3. Serial sections of each of the cochleae and the brain were studied in each of the animals.

4. In animals surviving section of the nerve for six weeks, serial section study revealed a complete degeneration of the cochlear ganglia with the nerve elements and efferent nerve fibers.

5. In every case following section of the nerve, even in cases which revealed complete cochlear ganglia degeneration on serial section, the cochlear response, when led off from the cochlea, was found to be unchanged, in so far as our type of apparatus might be expected to reveal changes.

6. Since the cochlear response persisted even after the complete degeneration of the nerve elements in the cochlear ganglia, it would seem that nervous elements in the cochlea, at least in the ganglia, play little if any rôle in the production of the cochlear response. The experiments reported in this paper throw no light on the question as to the living tissue or non-living structure origin of the response.

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THE RESPIRATORY EFFECT OF PROLONGED ANOXEMIA IN NORMAL DOGS BEFORE AND AFTER DENERVATION OF THE CAROTID SINUSES

C. L. GEMMILL, E. M. K. GEILING AND D. L. REEVES

From the Departments of Physiology, Pharmacology and Anatomy, The Johns Hopkins University, School of Medicine, Baltimore, Md.

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In a former paper Gemmill and Reeves (1933) reported the effect of acute anoxemia in normal dogs before and after carotid sinus denervation. They observed that the hyperpnea normally produced by administering nitrogen was abolished by carotid sinus denervation. Since one minute is the longest period normal dogs will tolerate the breathing of nitrogen there is the possibility that the respiratory or other centers may respond to an anoxemia less acute and more prolonged. In order to test this possibility it was decided to carry out a series of experiments on normal dogs in which acute and semi-acute asphyxial conditions were produced by two methods. The word "normal" is used in this connection to indicate that the dogs were neither under anesthesia nor the subjects of acute operations at the time of the experiments. In the first procedure the oxygen in the inspired air was reduced by administering mixtures of 14.5 per cent, 9.5 per cent and 5.1 per cent oxygen in nitrogen. Also, a study was made of the effects of breathing pure nitrogen. In the second method oxidations were decreased by intravenous injections and infusions of sodium cyanide solutions of such strengths as to produce *a*, an immediate effect, and *b* a delayed response. In this series, the dogs were submitted to most of the above-named procedures before and to all of the procedures after denervation of the carotid sinus regions. There was a slight variation in the composition of the various gas mixtures before and after denervation, due to the fact that it was necessary to make up new mixtures throughout the experiments.

METHODS. The procedures employed for administering the gas mixtures were similar to those described by Gemmill and Reeves (1933). The only change was the substitution of the rubber masks used by Geiling and DeLawder (1932) in place of the plaster of Paris masks. Denervation of the carotid sinuses was effected by complete removal of the bifurcation of the common carotid arteries (Gemmill and Reeves, 1933). Dog III was denervated on April 24, 1934, and dog IV on April 26, 1934. The weight of dog III was 13.0 kgm. and of dog IV, 12.0 kgm.

RESULTS. Preliminary experiments before operation were made on four

trained dogs. Unfortunately, at the time of operation, two dogs died of respiratory failure. As the respiration stopped suddenly in the expiratory phase, the respiratory death was very similar to that described by Witt, Katz and Kohn (1934). Therefore results after operation have been obtained on only two of the four dogs in this series.

Nitrogen. The results of one typical experiment before and after denervation of the carotid sinus regions are given in figure 1. The increase in depth of breathing and the resultant increase in minute volume following breathing of nitrogen was abolished by carotid sinus denervation. Occasionally, when the animals after denervation withstood breathing nitrogen for one minute an increase in rate of breathing was observed towards the end of the period.

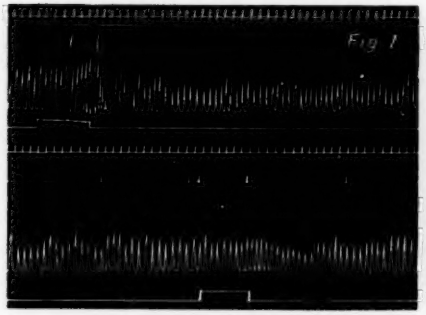
Five and one-tenth to 5.4 per cent O_2. These mixtures of oxygen in nitrogen were administered to the dogs for varying intervals of time before and after carotid sinus denervation. Results are given in figure 2. Before denervation the rate and amplitude increased during the first minute of breathing. After denervation there was only a slight change in rate during the first minute. During the second and third minutes, however, there occurred a marked change in rate, unaccompanied by an increase in depth of breathing. The minute volume increased in both cases, but the tidal air increased only in the experiment before denervation. The changes in rate observed in this experiment were the most marked of any noted in the series of experiments performed on the dogs after denervation. Generally there was only a slight or no increase in rate and minute volume.

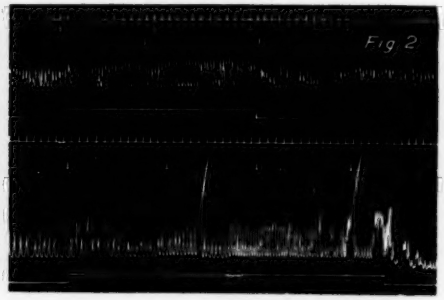
Nine and a half per cent O_2. Although the effects were not so marked, changes similar to those obtained with 5.1 per cent O_2 were also observed when the animals were subjected to 9.5 per cent O_2 (fig. 3). The increase in minute volume before denervation was again due to an increase both in rate and in depth, while after denervation the change was mainly due to an increase in rate, this change occurring at the end of the second minute of breathing the gas mixture.

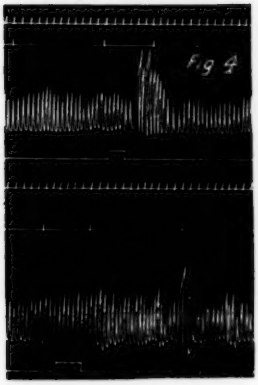
When the dogs were exposed to 14.5 per cent O_2, before denervation, the changes in respiration were so slight that these experiments were not repeated after denervation.

Cyanide. The response to the injection of 1 cc. of 0.03 molar sodium cyanide before denervation is given in figure 4. Twenty seconds after the injection the depth of breathing increased and remained elevated for eight respirations. The injection of twice this amount after denervation did not produce any marked change in depth but did give a slight increase in rate. The increase in rate increased slightly the minute volume. After denervation the two animals were also injected with pyruvic acid cyanohydrin, a substance recently studied by Marshall and Rosenfeld (1934) who have shown that it will produce a continuous respiratory response due to

the slow liberation of cyanide in the animal body. The dogs were also subjected to a continuous infusion of 0.01 molar sodium cyanide at the rate of 2 cc. per minute for twelve minutes. Both of these procedures were carried out under 0.5 grain morphine in order to prevent vomiting and







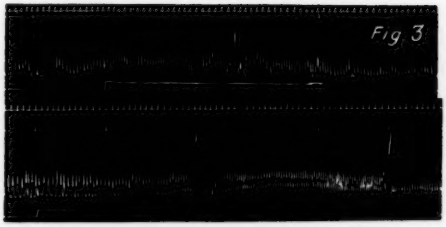


Fig. 1. Dog III. Upper line, five second intervals. Second line, time of meter readings. Third line, respiratory record. Fourth line, time of gas administration. Upper record, before denervation. Lower record, after denervation.

Fig. 2. Dog. III. Upper record, before denervation. Lower record, after denervation.

Fig. 3. Dog. III. Upper record, before denervation. Lower record, after denervation.

Fig. 4. Dog III. Upper record, before denervation. Lower record, after denervation.

gastric distress. In both instances there was a decided rise in minute volume. The increase with the injection of sodium cyanide came seven minutes after beginning the infusion. With pyruvic acid cyanohydrin the most marked effect was observed after a second injection of this substance.

In addition numerous experments were made on these dogs with injections of pitressin which is known to affect the respiration of normal dogs. Owing to the difficulty of interpreting the results, further experiments are necessary. They will be reported later.

Discussion. In their former work, Gemmill and Reeves (1933) observed that the respiratory effect of breathing nitrogen in normal dogs was abolished by denervation of the carotid sinuses. They concluded that the primary effect of anoxemia was on the nerve endings in the carotid sinuses. The present work confirms the previous observation (Gemmill and Reeves, 1933) that the respiratory response to acute anoxemia in normal dogs was abolished by denervation of the carotid sinus regions. In addition we have demonstrated that after the above named operative procedures the respiratory response to 2 cc. of 0.03 molar sodium cyanide solution injected rapidly was not obtained. However, when anoxemia was less acute and was prolonged over a longer period of time, a definite respiratory response was obtained. This was demonstrated by several methods, namely, anoxemia produced by decreasing the oxygen content of the inspired air, the injection of pyruvic acid cyanohydrin and the infusion of small amounts of sodium cyanide over a period of time. With the great individual variation from time to time in the respiratory response to breathing gas mixtures with low oxygen content an exact comparison of the responses before and after denervation cannot be made. It does appear, however, that after denervation the response is slower and is mainly a change of rate rather than of depth. Whether this effect is due to a direct or an indirect action on the respiratory centers in the brain or in some peripheral mechanism other than that of the carotid sinuses is left for future work to decide.

In acute experiments after carotid sinus denervation, Henderson and Greenberg (1934) have reported in abstract form that the administration of pure nitrogen produced no increase in breathing during the first 40 to 60 seconds of asphyxia. They did, however, observe an increase in ventilation when the asphyxia was continued into the second minute. Winder, Winder and Gesell (1933) have observed that trebling the dose of cyanide did produce a respiratory response after carotid sinus denervation and vagotomy. Their experiments and ours are in essential agreement.

SUMMARY

A study was made of the respiratory responses of four normal dogs before carotid sinus denervation and of two dogs after denervation to pure nitrogen, to gas mixtures of 14.5 per cent, 9.5 per cent and 5.1 per cent oxygen in nitrogen, and to cyanide injections.

The respiratory response to breathing pure nitrogen was abolished by denervation. After denervation the response to 9.5 per cent and 5.1 per

cent oxygen was absent during the first minute of breathing these mixtures. During the second and especially during the third minute there was an increase in rate of breathing without a corresponding increase in depth. No marked respiratory response was obtained from these dogs on breathing 14.5 per cent O_2.

The respiratory response to an immediately stimulatory dose of sodium cyanide was abolished by denervation. A definite stimulation, however, occurred when pyruvic acid cyanohydrin was injected, and also following the intravenous infusion of sodium cyanide over a period of time.

These experiments show that other mechanisms respond to respiratory stimulants after carotid sinus denervation. The response comes late and is more a change of rate than of depth. Thus the first reaction to acute anoxemia in unoperated dogs is through the carotid sinus mechanism, while the delayed response is probably through both the carotid sinus regions and other respiratory mechanisms.

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